SPECIES-RICH HEATHLANDS DEGRADED BY ATMOSPHERIC N DEPOSITION: PERSPECTIVES FOR RESTORATION

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SPECIES-RICH HEATHLANDS DEGRADED BY ATMOSPHERIC N DEPOSITION: PERSPECTIVES FOR RESTORATION

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Honderd wijzen zwegen toen er werd gevraagd,
Heeft elke vraag een antwoord
of is de vraag genoeg?

Rick de Leeuw

CONTENTS

Chapter 1

INTRODUCTION

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NITROGEN DEPOSITION

Nitrogen (N_2) is the most common gas in the earth's atmosphere. From the early 20th century, human activities such as urbanisation, the production of artificial fertilisers, burning of fossil fuels and intensive animal farming have resulted in severe disruption of N cycles on a global scale (see, e.g., Galloway, 1995; Galloway and Cowling, 2002; Van Egmond *et al.,* 2002). Anthropogenic emission of oxidised N (NO_y) and reduced N (NH_x) have increased drastically and at present account for a large part (65%) of the total global N budget, including natural sources (Olivier *et al.,* 1998). As a consequence, deposition of both NH_x and NO_y has increased. The annual input from atmospheric deposition was extremely high in the 1980s and early 1990s, reaching 30-40 kg ha^{-1} yr^{-1} in many areas of Central Europe and exceeding 60 kg ha^{-1} yr^{-1} in some others (Galloway, 1995).

Total N-deposition includes dry and wet deposition: dry deposition consists of gases such as NO_2 and NH_3 and fine particles (including fine particle NH_4^+), while wet deposition consists of NH_4^+, NO_3^- and NH_4^+-salts such as $(NH_4)_2SO_4$ and NH_4NO_3. Reduced nitrogen originates mainly from agricultural sources, whereas oxidised nitrogen is mainly emitted by energy production processes, industry and traffic (e.g. Galloway and Cowling, 2002). Deposition of NH_x in particular has been shown to be highest near its emission source (Erisman *et al.,* 1987; Ferm, 1998; Pitcairn *et al.,* 2002), whereas NO_y and especially NO_2 are transported over large distances (long-range transboundary pollutants). The deposition rate is not only influenced by the distance to the emission source and the deposition type, but also very much by the vegetation structure. Nitrogen deposition on vegetation with a rough canopy structure has been shown to be considerably higher than that on smooth surfaces (see, e.g., Van Breemen *et al.,* 1982).

Major differences in total N deposition rates and in NH_x and NO_y deposition exist between different regions. In countries such as the Netherlands, Belgium and Denmark with intensive animal farming, deposition mainly takes the form of reduced NH_x. In the air, NH_3 is readily converted to NH_4^+ and the deposition of NH_4^+ in these countries is very high. In contrast, the majority of deposited N in most Eastern European countries is in the oxidised NO_3^- form. Current deposition rates (total N deposition) in the Netherlands are among the highest in the world (up to 42 kg N ha^{-1} yr^{-1}; Eerens *et al.*, 2001).

EFFECTS OF N DEPOSITION

An increase in total N deposition drastically increases the availability of N in the soil, either directly or indirectly through processes such as increased mineralization, litter turnover rates and nitrification (see, e.g., Roelofs, 1986; Berendse, 1990; Van Vuuren, 1992; Aerts and Heil, 1993; Lee and Caporn, 1998; Bobbink *et al.,* 2003). Many natural and semi-natural ecosystems, including heathlands and mat grass swards, are naturally nutrient-poor (oligotrophic or mesotrophic) and N-limited (Gimingham, 1972; Bobbink *et al.,* 1992; Aerts and Heil, 1993). This has resulted in a high plant diversity with characteristic species adapted to N-limited, nutrient-poor conditions,

in which these species are able to survive and compete successfully with other species (**Figure 1**).

Figure 1: Species-rich grassland in Havelte, Drenthe, The Netherlands.

Increased N supply to oligotrophic and mesotrophic and N-limited systems stimulates plant productivity, as high N availability and mineralization rates enhance N uptake by plants. This has been shown to result in a decline of many characteristic species and an increase in highly competitive nitrophilic species (mainly grasses; see reviews by Fangmeier *et al.*, 1993; Pearson and Steward, 1993; Bobbink *et al.*, 1998; Krupa, 2003). The effect of increased atmospheric N deposition depends strongly on the total amount of accumulated N, the time of exposure to N input and the dominant form of N deposition (dry or wet, reduced or oxidised). In addition, abiotic soil conditions, plant-specific sensitivity and the type of management play important roles (Fangmeier *et al.*, 1993; Bobbink and Lamers, 2002, Bobbink *et al.*, 2003). Earlier work with individual plants showed that elevated NH_4^+ concentrations in soil solution can cause severe toxicity problems for many characteristic plant species of slightly buffered to acidic habitats, while in contrast, NO_3^- was shown to stimulate growth of most species (De Graaf *et al.*, 1998a; Britto and Kronzucker, 2002).

Increased NH_4^+ concentrations in the soil may result in increased uptake by plant roots and increased nitrification. Both processes produce protons and cause direct acidification of the soil. Acidification and processes related to acidification have been suggested as primary causes of the decline of herbaceous species in favour of grasses in slightly buffered and acid-sensitive ecosystems (Van Dam *et al.*, 1986; Dueck and

Elderson, 1992; Houdijk *et al.*, 1993). Rather than acidification, others have found the soil buffer range and acidification-related processes to be more predictive of the presence and absence and/or decline of herbaceous species (Houdijk *et al.,* 1993; Carnol *et al.,* 1997). The balance between acidified and slightly buffered systems is delicate and is referred to as the calcicole-calcifuge gradient (Balme, 1956; Hayati and Proctor, 1990; De Graaf, 2000). Following acidification, protons are exchanged for macronutrients such as calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K^{+}), thereby buffering the soil against acidifying processes (Scheffer and Schachtschabel, 1979). Subsequent leaching of Ca^{2+}, Mg^{2+} and K^{+} leads to loss of the soil's buffering capacity by base cations and to nutrient imbalance for plant growth (Houdijk *et al.*, 1993; Carnol *et al.*, 1997). A continuing process of acidification will lead to a shift in the buffer range of the soil from cation buffering (pH 4.5-6.0) to aluminium (Al^{3+}) buffering (pH < 4.5; Scheffer and Schachtschabel, 1979). Consequently, Al^{3+} and micronutrients such as manganese (Mn^{2+}) and iron (Fe^{2+}, Fe^{3+}) are exchanged for protons, resulting in increased concentrations in the soil. Earlier studies have shown that Al^{3+} is toxic to many plant species (see, e.g., Ryan *et al.,* 1994; Kinraide, 1997). Fennema (1992), Heijne *et al.* (1996) and De Graaf *et al.* (1997) attributed the decline of many characteristic plant species in species-rich acidic grasslands and heathlands to Al-toxicity effects and increased Al/Ca ratios in the soil as a result of increased acidification.

In general, NH_4^+-tolerant species are found in acidic habitats and are likely to be adapted to NH_4^+ nutrition, since NH_4^+ is the dominant form of N at low pH. In contrast, species from more buffered habitats usually prefer NO_3^- (Gigon and Rorison, 1972; Falkengren-Grerup, 1995; Troelstra *et al.*, 1995; Britto and Kronzucker, 2002). Despite the plants' preferences, NH_4^+ is readily taken up by both NH_4^+-tolerant and NH_4^+-intolerant species, as NH_4^+ uptake is energetically more favourable than NO_3^- uptake. Following uptake, NH_4^+ has to be assimilated immediately, as it causes severe toxicity symptoms in plants (see, e.g., Mehrer and Mohr, 1989; Britto and Kronzucker, 2002, and references therein). In a hydroponic experiment with three characteristic species from species-rich heathlands and grasslands (*Arnica montana*, *Cirsum dissectum* and *Calluna vulgaris*), NH_4^+ toxicity symptoms were shown to occur above a concentration of 100 µmol l^{-1} in both of the herbaceous species, whereas *Calluna vulgaris* performed well at NH_4^+ concentrations up to 1000 µmol l^{-1} (De Graaf *et al.,* 1998a). NH_4^+ toxicity effects were found to be expressed mainly as Mg^{2+} and K^{+} deficiencies (De Graaf *et al.,* 1998a). Similar effects were found in another study, in which NH_4^+ uptake was shown to prevent cation uptake and cause cation deficiencies when plants were grown with NH_4^+ as their sole N source (Roelofs *et al.,* 1985).

With increasing N loads at high deposition rates, the oligotrophic and/or mesotrophic ecosystems shift from N limitation towards P limitation. Ten Harkel and Van der Meulen (1995) found substantial NO_3^- leaching (4-11 kg N ha^{-1} yr^{-1}) from grassland soils to which 25-50 kg N ha^{-1} yr^{-1} was added, indicating a surplus of N in the soil. They suggested that P limitation occurs after several decades of high N deposition. In addition, P limitation was shown to affect plant production and, subsequently, species composition in calcareous grasslands (Morecroft *et al.,* 1994;

Carroll *et al.,* 2003) and in calcareous dune grasslands (Kooijman and Besse, 2002). With increased N availability, the N content in this P-limited vegetation was shown to increase (Baddeley *et al.,* 1994; Pitcairn *et al.,* 1995) and this was suggested to affect the plants' susceptibility to herbivores (heather beetle) and pathogens, as well as mycorrhizal infection rates and sensitivity to frost and drought (Aerts and Heil, 1993; Bobbink and Heil, 1993; Power *et al.,* 1998; Carroll *et al.,* 1999).
The effects of atmospheric N deposition on vegetation development have been extensively investigated (see, e.g., Bobbink and Heil, 1993; Power *et al.*, 1998; Caroll *et al.*, 2003; Falkengren-Grerup and Schöttelndreier, 2004). Despite the large differences in reduced and oxidised N deposition within Europe and the potential for different ecological impacts, very few experimental or field studies have rigorously assessed the differential effects of reduced and oxidised N and even fewer have considered the importance of interactions with soil acidity. The importance of such studies is however widely recognised (Bobbink *et al.,* 1998; 2003). A comparative study by Smart *et al.* (2004) showed a clear correlation between changes in the vegetation of species-rich grasslands and heathlands and atmospheric deposition of reduced N, whereas no significant correlation was found with the deposition of oxidised N. In an N manipulation experiment, Lütke Twenhöven (1992) reported various effects of NH_4^+ and NO_3^- deposition on bog vegetation. Others have also investigated the effects of different NH_4^+/NO_3^- ratios in N deposition on vegetations (Schuurkens, 1984; Roelofs, 1986; Paulissen *et al.*, 2004). Comparative studies using fixed N deposition rates but varying NH_4^+/NO_3^- ratios investigating interactions between NH_4^+/NO_3^- ratios, soil processes and biogeochemical parameters are needed as it is important to understand how and to what extent these processes affect the vegetation development.

RESTORATION OF HEATHLANDS AND SPECIES-RICH GRASSLANDS

The sharp decline in species diversity, as a result of atmospheric deposition of acidifying and eutrophying compounds, has made it urgent for policy makers, landowners and managers to develop and apply specific and effective restoration measures. Traditional management such as grazing by sheep and large herbivores (which has been used from as early as 3000 BC) and turf removal (sod cutting; which prevailed in the Middle Ages) resulted in the creation of large areas of heathland and species-rich grasslands until the mid-nineteenth century (De Smidt, 1979; **Figure 2**).
Since the 1980s, these management practices have been widely applied as measures to reduce grass encroachment and nutrient accumulation in the soil and to restore degraded heathlands and species-rich grasslands. Although grazing proved effective against grass encroachment, the removal of accumulated nutrients by grazing is a long-term process (Armstrong *et al.*, 1997). In addition to reducing the dominance of grasses and the accumulation of nutrients, sod cutting creates opportunities for the establishment and germination of many endangered herbaceous species (Bakker, 1989; Aerts and Heil, 1993; Snow and Marrs, 1997). In the early 1990s, additional restoration measures were applied to counteract site-specific problems. Among these are lime addition to counteract the effects of acidification and restoration of former

hydrology to counteract the negative effects of drainage (De Graaf *et al.,* 1998b; Roelofs *et al.,* 1996).

Figure 2: Heathland in Havelte, Drenthe, grazed by sheep.

Experiments in acidic grasslands and heathlands to assess the effects of lime addition and sod cutting on the vegetation development and vegetation recovery have shown that liming successfully restores the soil's pH and buffering capacity. In addition, nutrient concentrations were reduced by sod cutting. Nevertheless, vegetation development and the germination of characteristic herbaceous species, like *A. montana*, remained below expectations (De Graaf *et al.,* 1998a). De Graaf *et al* (1998a) and Dorland *et al.* (2003) have shown that this poor development and the decline of many herbaceous species could be attributed to high Al^{3+} and high NH_4^+ concentrations in the first 1-2 years after sod cutting. Suggested causes of this accumulation of NH_4^+ include enhanced mineralization and the absence of NH_4^+ uptake. The recovery of the characteristic vegetation in these systems is thus limited by biogeochemical factors. In addition, most of the characteristic herbaceous species have a non-persistent seed bank (< 5 years), which makes them vulnerable to poor soil conditions (Bakker *et al.,* 1996). Moreover, removing the organic top layer means that many of the micro-organisms and organic compounds in the ecosystem are removed as well. The effects of this change on the development of the vegetation and on various soil processes are largely unknown. Moreover, since it has been shown that in most of the acidic and acid-sensitive vegetation types, NH_4^+ accumulates even after restoration measures such as sod cutting, it is important to examine the responses of specific plants to high NH_4^+ concentrations and to altered NH_4^+/NO_3^- ratios.

Aim of the studies

The aim of the studies reported on in this thesis was to investigate possible constraints on the restoration and conservation of species-rich Nardo-Gallion grasslands, stable dune grasslands and heathlands. The thesis focuses on interactions between biogeochemical parameters and biogeochemical processes in the soil and vegetation responses and vegetation development. More specifically, it discusses the

effects of N deposition levels, high NH_4^+ concentrations, various NH_4^+/NO_3^- ratios, acidification and restoration measures such as lime addition. The following research questions are addressed:

General research questions

- What are the effects of elevated N deposition on the development and composition of nutrient-poor vegetation types?
- What are the effects of different NH_4^+/NO_3^- ratios on the development and composition of nutrient-poor vegetation types?
- What direct or indirect roles does acidification play in the vegetation development, by enhancing NH_4^+ toxicity and by affecting biogeochemical parameters and microbial processes in the soil?
- What are the biogeochemical consequences of the application of restoration measures such as sod cutting and liming?

Outline of the thesis

Chapter 2 of this thesis discusses critical N loads for dune grasslands. In this study we used a large mesocosm set-up to investigate and establish empirical critical N loads for this vegetation type. N deposition was varied in this experiment by applying a range of NH_4NO_3 concentrations, resulting in N deposition rates of 1, 10, 20, 40, 60 and 80 kg ha^{-1} yr^{-1}. Plants of two graminoid species (*Calamagrostis epigejos* and *Carex arenaria*) and two herbaceous species (*Galium verum* and *Carlina vulgaris*) were planted in the mesocosms, which were filled with sand originating from stable dune grassland. Growth and development of these species were recorded, as was the nutritional status of the plants.
Chapter 3 reports on a study into the effects of NH_4^+ on the growth and survival of five different plant species in a slightly buffered and an acidified situation. Using hydroponic techniques, we grew the acid-sensitive species *Antennaria dioica*, *Succisa pratensis* and *Gentiana pneumonanthe* (which are declining in many Dutch heathlands and mat grass swards) as well as a more common species, *Calluna vulgaris* and a highly competitive grass species, *Deschampsia flexuosa*. This study was carried out in close collaboration with Edu Dorland of Utrecht University, The Netherlands.
The effect of increasing NH_4^+/NO_3^- ratios in the soil is analysed in **chapter 4**, which reports on a study using large mesocosms. The mesocosms (1 x 1 m) were filled with sand from heathland, from which the topsoil had been removed. The grasses *D. flexuosa*, *Nardus stricta* and *Danthonia decumbens*, the herbaceous species *G. pneumonanthe*, *A. montana*, *Thymus serpyllum* and *A. dioica*, and the shrub *Calluna vulgaris* were planted in the mesocosms, in which different NH_4^+/NO_3^- ratios were created by manipulating N deposition. In order to investigate the effects of lime addition and hence the interaction with soil pH, half of the mesocosms received a lime treatment (200 g m^{-2}).

Restoration measures such as turf cutting and lime addition may significantly affect the biogeochemistry of the soil. To test whether the removal of the top layer affects the nitrifying capacity of the soil and whether this affects the conversion of NH_4^+ to NO_3^-, an experiment was conducted in collaboration with Edu Dorland of Utrecht University, in which potential nitrification was measured in heathland soil before and after turf cutting (**chapter 5**). The effect of lime addition to these soils was also measured, as lime addition increases soil pH, which was shown to increase nitrification activity.

Chapter 6 reports on a study investigating the effects of turf cutting on the germination of the herbaceous species *A. montana* in relation to Al toxicity. Application of lime was tested because increased pH and Ca concentrations were expected to result in a shift towards cation buffering and, hence, reduction of Al toxicity. The study also addressed questions concerning the effects of turf cutting on the removal of the organic top layer, including most of the humic acids and hence on the soil's capacity to buffer free Al^{3+} ions.

The final chapter (**chapter 7**) discusses the results of the preceding chapters, and considers the consequences of these findings for the restoration of heathlands and mat grass swards.

REFERENCES

Aerts, R., Heil, G.W. 1993. *Heathlands: patterns and processes in a changing environment*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Armstrong, H.M., Gordon, I.J., Hutchings, N.J., Illius, A.W., Milne, J.A., Sibbald, A.R. 1997. A model of the grazing of hill vegetation by sheep in the UK. II. The prediction of offtake by sheep. *Journal of Applied Ecology*, 34: 186-207.

Baddeley, J.A., Woodin, S.J., Alexander, I.J. 1994. Effects of increased nitrogen and phosphorus availability on the photosynthesis and nutrient relations of three arctic dwarf shrubs from Svalbard. *Functional Ecology*, 8: 676-685.

Bakker, J.P. 1989. *Nature management by grazing and cutting*. Academic Press Publishers, Dordrecht, The Netherlands.

Bakker, J.P., Podschlod, P., Strykstra, R.J., Bekker, R.M., Thompson, K. 1996. Seed banks and seed dispersal: important topics in restoration ecology. *Acta Botanica Neerlandica*, 45: 461-490.

Balme, O.E. 1956. Edaphic and vegetational zoning on the carboniferous limestone of the Derbyshire Dales. *Journal of Ecology*, 41: 331-344.

Berendse, F. 1990. Organic matter accumulation and nitrogen mineralization during secondary succession in heathland ecosystems. *Journal of Ecology*, 78: 413-427.

Bobbink, R., Ashmore, M., Braun, S., Flückiger, W., Van den Wyngaert, I.J.J. 2003. Empirical nitrogen critical loads for natural and semi-natural ecosystems: 2002 update. In: Achermann, B., Bobbink, R. (Eds.). *Empirical critical loads for nitrogen - Proceedings*. SAEFL, Berne, Switzerland, pp. 43-170.

Bobbink, R., Boxman, A.W., Fremstad, E., Heil, G., Houdijk, A.L.F.M., Roelofs, J.G.M. 1992. Critical loads for nitrogen eutrophication of terrestrial and wetland ecosystems based upon changes in vegetation and fauna. In: Grennfelt, P., Thörnelöf, E. (Eds.). *Critical loads for nitrogen.* Nordic Council of Ministers, Copenhagen, Denmark, pp. 111-159.

Bobbink, R., Heil, G.W. 1993. Atmospheric deposition of sulphur and nitrogen in heathland

ecosystems. In: R. Aerts, G.W. Heil (Eds.). *Heathlands: patterns and processes in a changing environment. Geobotany 20.* Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 25-50.

Bobbink, R., Hornung, M., Roelofs, J.G.M. 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, 86: 717-738.

Bobbink, R., Lamers, L.P.M. 2002. Effects of increased nitrogen deposition. In: Bell, J.N.B., Treshow, M. (Eds.). *Air pollution and plant life* (2nd edition). John Wiley and Sons, Chichester, United Kingdom, pp 201-235.

Britto, D.T., Kronzucker, H.J. 2002. NH_4^+ toxicity in higher plants: a critical review. *Journal of Plant Physiology*, 159: 567-584.

Carnol, M., Ineson, P., Dickinson, A.L. 1997. Soil solution nitrogen and cations influenced by $(NH4)_2SO_4$ deposition in a coniferous forest. *Environmental Pollution*, 97: 1-10.

Carroll, J.A., Caporn, S.J.M., Cowley, L., Read, D.J., Lee, J.A. 1999. The effects of increased deposition of atmospheric nitrogen on *Calluna vulgaris* in upland Britain. *New Phytologist*, 141: 423-431.

Carroll, J.A, Caporn, S.J.M., Johnson, D., Morecroft, M.D., Lee, J.A. 2003. The interactions between plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition. *Environmental Pollution*, 121: 363-376.

De Graaf, M.C.C., Bobbink, R., Verbeek, P.J.M., Roelofs, J.G.M. 1997. Aluminium toxicity and tolerance in three heathland species. *Water, Air and Soil Pollution*, 98: 229-241.

De Graaf, M.C.C., Bobbink, R., Roelofs, J.G.M., Verbeek, P.J.M. 1998a. Differential effects of ammonium and nitrate on 3 heathland species. *Plant Ecology*, 135: 185-196.

De Graaf, M.C.C., Verbeek, P.J.M., Bobbink, R., Roelofs, J.G.M. 1998b. Restoration of species-rich dry heaths: the importance of appropriate soil conditions. *Acta Botanica Neerlandica*, 47: 89-113.

De Graaf, M.C.C. 2000. *Exploring the Calcicole - Calcifuge gradient in heathlands.* PhD Thesis, University of Nijmegen, Nijmegen, The Netherlands.

De Smidt, JT. 1979. Origin and destruction of Northwest European heath vegetation. In: Wilmanns, O., Tüxen, R. (Eds.). *Werden und Vergehen von Pflanzengesellschaften*. J. Cramer, Vaduz, Liechtenstein, pp. 411-435.

Dorland, E., Bobbink, R., Messelink, J.H., Verhoeven, J.T.A. 2003. Soil ammonium accumulation after sod cutting hampers the restoration of degraded wet heathlands. *Journal of Applied Ecology*, 40: 804-814.

Dueck, T.A., Elderson, J. 1992. Influence of NH_3 and SO_2 on the growth and competitive ability of *Arnica montana* L. and *Viola canina* L. *New Phytologist*, 122: 507-512.

Eerens, H.C., Van Dam, J.D., Beck, J.P., Dolmans, J.H.J., Van Pul, W.A.J., Sluyter, R.B.C., Van Velze, K., Vissenberg, H.A. 2001. *Grootschalige luchtverontreiniging en depositie in de Nationale Milieuverkenning 5*. RIVM report 408129016/2001. Bilthoven, The Netherlands.

Erisman, J.W., Vermetten, A.W.M., Pinksterboer, E.F., Asman, W.A.H., Waijers-Ypelaan, A., Slanina, J. 1987. Atmospheric ammonia: distribution, equilibrium with aerosols and conversion rate to ammonium. In: Asman, W.A.H., Diederen, H.S.M.A. (Eds.). *Ammonia and acidification*, Symposium EURASAP, Bilthoven, The Netherlands, 13-15 April 1987. RIVM/TNO, Bilthoven, pp. 59-72.

Falkengren-Grerup, U. 1995. Interspecies differences in the preference of ammonium and nitrate in vascular plants. *Oecologia*, 102: 305-311.

Falkengren-Grerup, U., Schöttelndreier, M. 2004. Vascular plants as indicators of nitrogen enrichment in soil. *Plant Ecology*, 172: 51-62.

Fangmeier, A., Hadwiger-Fangmeier, A., Van der Eerden, L., Jäger, H-J. 1993. Effects of

atmospheric ammonia on vegetation - a review. *Environmental Pollution*, 86: 43-82.
Fennema, F. 1992. SO_2 and NH_3 deposition as possible cause for the extinction of *Arnica montana* L. *Water, Air and Soil Pollution*, 62: 325-336.
Ferm, M. 1998. Atmospheric ammonia and ammonium transport in Europe and critical loads: a review. *Nutrient Cycling in Agroecosystems*, 51: 5-17.
Galloway, J.N. 1995. Acid deposition: perspectives in time and space. *Water, Air and Soil Pollution*, 85: 15-24.
Galloway, J.N., Cowling, E.B. 2002. Reactive nitrogen and the world: 200 years of change. *Ambio*, 31(2): 64-71.
Gigon, A., Rorison, I.H. 1972. The response of some ecologically distinct plant species to nitrate- and to ammonium-nitrogen. *Journal of Ecology*, 60: 93-102.
Gimmingham, C.H. 1972. Ecology of Heathlands. Chapman and Hall, London, United Kingdom.
Hayati, A.A., Proctor, M.C.F. 1990. Plant distribution in relation to mineral nutrient availability and uptake on a wet-heath site in south-west England. *Journal of Ecology*, 78: 134-151.
Heijne, B., van Dam, D., Heil, G.W., Bobbink, R. 1996. Acidification effects on vescular-arbuscular mycorrhizal (VAM) infection, growth and nutrient uptake of established heathland herb species. *Plant and Soil*, 179: 197-206.
Houdijk, A.L.F.M., Verbeek, P.J.M., Van Dijk, H.F.G., Roelofs, J.G.M. 1993. Distribution and decline of endangered heathland species in relation to the chemical composition of the soil. *Plant and Soil*, 148: 137-143.
Kinraide, T.B. 1997. Reconsidering the rhizotoxicity of hydroxyl, sulphate and fluoride complexes of aluminium. *Journal of Experimental Botany*, 48: 1115-1124.
Kooijman, A.M., Besse, M. 2002. The higher availability of N and P in lime-poor than in lime-rich coastal dunes in the Netherlands. *Journal of Ecology*, 90: 394-403.
Krupa, S.V. 2003. Effects of atmospheric ammonia (NH_3) on terrestrial vegetation: a review. *Environmental Pollution*, 124: 179-221.
Lee, J.A., Caporn, S.J.M. 1998. Ecological effects of atmospheric reactive nitrogen deposition on semi-natural terrestrial ecosystems. *New Phytologist*, 139: 127-134.
LütkeTwenhöven, F. 1992. Competition between two *Sphagnum* species under different deposition levels. *Journal of Bryology*, 17: 71-80.
Mehrer, I., Mohr ,H. 1989. Ammonium toxicity: description of the syndrome in *Sinapis alba* and the search for its causation. *Physiologia Plantarum*, 77: 545-554.
Morecroft, M.D., Sellers, E.K., Lee, J.A. 1994. An experimental investigation into the effects of atmospheric nitrogen deposition on two semi-natural grasslands. *Journal of Ecology*, 82: 475-483.
Olivier, J.G.J., Bouwman, A.F., Van der Hoek, K.W., Berdowski, J.J.M. 1998. Global air emission inventories for anthropogenic sources of NO_x, NH_3 and N_2O in 1990. *Environmental Pollution*, 102: 135-148.
Paulissen, M.P.C.P., van der Ven, P.J.M., Dees, A.J., Bobbink, R. 2004. Differential effects of nitrate and ammonium on three fen bryophyte species in relation to pollutant nitrogen input. *New Phytologist*, 164(3): 451-458.
Pearson, J., Stewart, G.R. 1993. Tansley Review No 56: The deposition of atmospheric ammonia and its effects on plants. *New Phytologist*, 125: 283-305.
Pitcairn, C.E.R., Fowler, D., Grace, J. 1995. Deposition of fixed atmospheric nitrogen and foliar nitrogen content of bryophytes and *Calluna vulgaris*. *Environmental Pollution*, 88: 193-205.
Pitcairn, C.E.R., Skiba, U.M., Sutton, M.A., Fowler, D., Munro, R., Kennedy, V. 2002. Defining the spatial impact of poultry farm ammonia emissions on species composition of

adjacent woodland ground flora using Ellenberg nitrogen Index, nitrous oxide and nitric oxide emissions and foliar nitrogen as marker variables. *Environmental Pollution*, 119: 9-21.

Power, S.A., Ashmore, M.R., Cousins, D.A., Sheppard, L.J. 1998. Effects of nitrogen addition on the stress sensitivity of *Calluna vulgaris*. *New Phytologist*, 138: 663-673.

Roelofs, J.G.M. 1986. The effect of airborne sulphur and nitrogen deposition on aquatic and terrestrial heathland vegetation. *Experientia*, 42: 372-377.

Roelofs, J.G.M., Bobbink, R., Brouwer, E., De Graaf, M.C.C. 1996. Restoration ecology of aquatic and terrestrial vegetation on non-calcareous sandy soils in the Netherlands. *Acta Botanica Neerlandica*, 45: 517-541.

Roelofs, J.G.M., Kempers, A.J., Houdijk, L.F.M., Jansen J. 1985. The effect of air-borne ammonium sulphate on *Pinus nigra* var. *maritima* in the Netherlands. *Plant and Soil*, 84: 45-56.

Ryan, P.R., Kinraide, T.B., Kochian, L.V. 1994. Al^{3+}-Ca^{2+} interactions in aluminium rhizotoxicity. I. Inhibition of root growth is not caused by reduction of calcium uptake. *Planta*, 192: 98-103.

Scheffer, F., Schachtschabel, P. 1979. *Lehrbuch der Bodenkunde*. Ferdinand Enke Verlag, Stuttgart, Germany.

Schuurkens, J.A.A.R., Elbers, M.A., Gudden, J.J.F., Roelofs, J.G.M. 1987. Effects of simulated ammonium sulphate and sulphuric acid rain on acidification, water quality and flora of small-scale soft water systems. *Aquatic Botany*, 28: 199-226.

Smart, S.M., Ashmore, M.R., Hornung, M., Scott, W.A., Fowler, D.A., Dragosits, U., Howard, D.C., Sutton, M.A., Famulari, D. 2004. Detecting the signal of atmospheric N deposition in recent national-scale vegetation change across Britain. *Water, Air and Soil Pollution: Focus*, 4: 296-278.

Snow, C.S.R., Marrs, R.H. 1997. Restoration of *Calluna* heathland on a Bracken *Pteridium* - infested site in North West England. *Biological Conservation*, 81: 35-42.

Ten Harkel, M.J., Van der Meulen, F. 1995. Impact of grazing and atmospheric nitrogen deposition on the vegetation of dry coastal dune grasslands. *Journal of Vegetation Science*, 6: 445-452.

Troelstra, S.R., Wagenaar, R., Smant, W. 1995. Nitrogen utilization by plant species from acid heathland soils. I. Comparison between nitrate and ammonium nutrition at constant low pH. *Journal of Experimental Botany*, 46: 1103-1112.

Van Breemen, N., Burrough, P.A., Velthorst, E.J., van Dobben, H.F., de Wit, T., Ridder, T.B., Reijnders, H.F.R. 1982. Soil acidification from atmospheric ammonium sulphate in forest canopy throughfall. *Nature*, 299: 548-550

Van Dam, D., Van Dobben, H.F., Ter Braak, C.F.J., De Wit, T. 1986. Air pollution as a possible cause for the decline of some phanerogamic species in the Netherlands. *Vegetatio*, 65: 47-52.

Van Egmond, K., Bresser, T., Bouwman, L. 2002. The European Nitrogen case. *Ambio*, 31(2): 72-78.

Van Vuuren, M. 1992. *Effects of plant species on nutrient cycling in heathlands*. PhD Thesis, Utrecht University, Utrecht, The Netherlands.

Chapter 2

EFFECTS OF NITROGEN ENRICHMENT ON COASTAL DUNE GRASSLAND: A MESOCOSM STUDY

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SUMMARY Mesocosms filled with dune sand were planted with graminoid (*Calamagrostis epigejos*, *Carex arenaria*) and herbaceous species (*Carlina vulgaris*, *Galium verum*). Strong effects of nitrogen addition on the vegetation were found within two to three years. The above-ground biomass of *C. epigejos* and *C. arenaria* increased at deposition rates between 10 and 80 kg N ha^{-1} yr^{-1}. Both graminoids were limited by N. In latter stages P-limitation was suggested for *C. arenaria*. At high N-levels, *C. epigejos* dominated the vegetation within two years. *Carlina vulgaris* and *G. verum* declined drastically as a result of increased competition for light by the highly competitive grass *C. epigejos*. It is concluded that increased (ambient) N inputs are of major importance for the increased dominance of tall grasses in stable dune grasslands.

INTRODUCTION

During the past decades, the structure and functioning of several ecosystems in densely populated countries have been threatened by high inputs of air-borne nitrogen (N; see Krupa, 2003 and references therein). In contrast to the estimated background inputs of 1-5 kg N ha^{-1} yr^{-1} in the early 1900s, present N deposition rates can amount to 20-60 kg ha^{-1} yr^{-1} in non-forest ecosystems in NW Europe (e.g. Erisman, 1993; Bobbink and Heil, 1993; Asman *et al.*, 1998; Harriman *et al.*, 1998). Recently it has been shown that annual deposition rates of N also strongly increased in other parts of the world, including areas in India and China (Galloway, 2000; Galloway and Cowling, 2002).

N frequently limits plant growth in many oligotrophic ecosystems. Enhanced N deposition was shown to result in grass encroachment and the loss of herbaceous species in calcareous grasslands (e.g. Bobbink and Willems, 1987; Carroll *et al.*, 2003) and dry acidic grasslands (Bobbink *et al.*, 1996; Berlin *et al.*, 2000). Grass encroachment was also found in species-rich, relatively open grasslands in the coastal dunes of the Netherlands (Veer and Kooijman, 1997). Today, *Ammophila arenaria* is the dominant species in the primarily non-calcareous dunes in the coastal area in NW Netherlands, whereas in more calcareous areas, the grasses *Calamagrostis epigejos, Elymus repens* and *Elymus athericus* dominate (Kooijman and De Haan, 1995; Kooijman and Van der Meulen, 1996). The present dominance of tall grasses in the coastal dunes in the Netherlands may be explained by an increase in nutrients in the soil due to atmospheric N deposition. In addition, changes in management practices, the impact of grazing (mainly by rabbits), may partly explain the shifts in vegetation composition. It is known that traditional management using large grazers (Klapwijk, 1996; Arens *et al.*, 2000) and grazing by rabbits significantly reduces the dominance of tall grasses in grasslands (Williams *et al.*, 1974; Jutila, 2002). Ten Harkel and Van der Meulen (1995) have shown that exclusion of grazing in areas with high N deposition resulted in dominance of tall grasses within one to two years.

Fertilisation experiments in different types of grasslands have shown grass encroachment with increased N availability, indicating N limitation (e.g. Boorman and Fuller, 1982; Bobbink *et al.*, 1988; Bobbink, 1991; Berlin *et al.*, 2000). N limitation was also observed in coastal dune grasslands in the Netherlands, as well as across England and Wales (Kooijman *et al.*, 1998; Jones *et al.*, 2004). In contrast, Ten Harkel and Van der Meulen (1995) found substantial nitrate (NO_3^-) leaching (4-11 kg N ha^{-1} yr^{-1}) from grassland soils to which 25-50 kg N ha^{-1} yr^{-1} was added and suggested P limitation after several decades of high N deposition. In addition, P limitation was shown to affect species composition in (calcareous) grasslands (Morecroft *et al.*, 1994; Carroll *et al.*, 2003) and in calcareous dune grasslands (Kooijman and Besse, 2002). Vegetation responses in coastal dune ecosystems may thus be explained by effects of atmospheric N deposition and the chemical status (N limitation or P limitation or both) of the soil.

Empirical critical loads for N deposition have been developed to quantify the sensitivity of ecosystems to the impacts of N deposition. These have been derived from significant changes in the structure or function of ecosystems using experimental (field) data, field observations and/or dynamic ecosystem models

(Bobbink and Roelofs, 1995; Bobbink *et al.*, 2003). Based upon field data (grass encroachment at > 15 kg N ha^{-1} yr^{-1} and NO_3^- leaching at > 20 kg N ha^{-1} yr^{-1}), critical loads for coastal stable dune grasslands and shifting coastal dunes are recently set at 10-20 kg N ha^{-1} yr^{-1}, respectively (Bobbink *et al.*, 2003). There is a strong need for these critical load values to be validated using N-manipulating experiments. However, such experiments are sparse for dune grassland-systems and have not yet been performed at a low background deposition.
The aim of this study is to obtain experimental evidence for the estimated critical N loads for coastal stable dune grasslands. Therefore, plants from both grass species (*C. epigejos* L. Roth. and *Carex arenaria* L.) and endangered herbaceous species (*Galium verum* L. and *Carlina vulgaris* L.) were planted in mesocosms filled with nutrient poor soil from a calcareous dune grassland to mimic a nutrient poor primary dune slack. During a three-year experimental period, different N loads were simulated in order to analyse the effect of N deposition on the growth of these species. We hypothesised that biomass and cover of both grass species increased drastically with increasing N deposition, whereas the herbaceous species declined strongly as a result of this grass encroachment.

MATERIALS AND METHODS

On 9 June 1997, 5 m^3 dune sand was collected from a calcareous dune grassland in Oostvoorne at the Dutch coast (51°54'N, 4°04'E). Nutrient poor sand was collected in an approximately 90-year old primary dune slack, from which the organic top layer was removed to reduce the N-content in the soil. Sand was collected to a depth of maximum 30 cm. Twelve polyethylene containers (1.0 x 1.0 x 0.4 m) equipped with drainage openings, were filled with this sand and placed in a greenhouse. The greenhouse was not heated to approach natural seasonal variances in temperature and humidity (mean temperature was 15°C and air humidity 75%). The greenhouse was however climate controlled by shields and ventilation, resulting in average temperature differences of no more than 3.3 degrees Celsius with the lower temperature outside the greenhouse. The background deposition (dry N-deposition measured for two years using funnels) in the greenhouse was approximately 1 kg N ha^{-1} yr^{-1}. In order to analyse soil pore water, three soil moisture samplers (Rhizon SMS-10 cm; Eijkelkamp Agrisearch Equipment) were placed in every mesocosm at a depth of 10-15 cm. Soil pore water was collected by connecting a vacuum infusion flask (100 ml) to each sampler. The samples were stored at 4°C (for a maximum of 2 weeks) until further analysis. Six treatments (n = 2) were randomly applied, differing in N concentration and leading to N loads of 1, 10, 20, 40, 60 and 80 kg N ha^{-1} yr^{-1}, respectively. N was added as ammonium nitrate (NH_4NO_3) and annual amount of artificial rainwater was 500 mm. The applied artificial rainwater contained 5 mg l^{-1} sea salt ('Marine mix + Bio-elements', Wiegandt GmbH, FRG) and 13 µmol l^{-1} $NaH_2PO_4 \cdot H_2O$ and was supplied twice a week using a watering can.
The mesocosms were pre-treated for a period of ca. 2 months with N-free demineralised water containing 5 mg l^{-1} sea salt (total precipitation 235 mm). Pre treatment was necessary in order to remove the surplus of NO_3^- in the soil, due to

increased mineralization after filling. When NO_3^- concentrations in soil pore water reached values below 10 µmol l^{-1}, the N addition started. At this moment (August 1997) plants were introduced into the mesocosms. In each mesocosm 35 plants of *C. epigejos,* 25 plants of *C. arenaria,* 4 plants of *Carlina vulgaris* and 10 plants of *G. verum* were planted as to represent a natural vegetation composition. *C. epigejos, C. arenaria* and *G. verum* plants were obtained from cuttings of local sods (Oostvoorne, annual deposition of ca. 15-20 kg N ha^{-1} yr^{-1}). *Carlina vulgaris* plants were seedlings grown from commercially derived seeds, originating from wild plants (Blauetikett Borntraeger GmbH, Offstein, Germany). The shoots of *C. epigejos* and *C. arenaria* were cut back to 10 cm height. Plants were randomly planted in each mesocosm. In November 1997 and April 1998, the algal material in the top soil (0-0.5 cm) was measured via active chlorophyll-a concentration. Therefore, five sub samples were collected from each mesocosms using an auger (diameter 3 cm), after which chlorophyll was extracted with 80% ethanol at 70°C. After cooling and centrifugation, light absorption was measured at 665 and 750 nm. The amount of active chlorophyll-a could be calculated by correction for the amount of phaeopigments (degraded chlorophyll). After two years from the start of the experiment, the above-ground and below-ground biomass (48 hours at 70°C) for all species was determined in one half of each mesocosms. After three years the above-ground and below-ground biomass was determined, using the same method, for the species in the other half of each mesocosm. Total grass biomass was measured as the sum of the biomass of *C. epigejos* and *C. arenaria*.

Chemical analysis

N concentrations in below-ground material and above-ground shoots of *C. epigejos* and *C. arenaria* were measured after two years and at the end of the experiment. Due to lack of plant material, the N-concentrations could not be measured for the species *Carlina vulgaris* and *G. verum*. The concentrations of Ca, Mg, K, Mn and P in the below-ground and above-ground material of *G. verum*, *C. epigejos* and *C. arenaria* were measured at the end of the experiment. For analyses of the chemical composition of plant material, dried plant material (48 hrs 70°C) were ground in liquid N. Hundred mg of ground plant material was digested in sealed Teflon vessels in a Milestone destruction microwave oven (MLS 1200 mega) with nitric acid and hydrogen peroxide. Plant material was analysed for Ca, Mg, Mn and P using an inductively-coupled plasma emission spectrophotometer (ICP, Spectroflame Flame VML2). K was determined with a flame photometer, using a Technicon I Auto Analyser (Technicon, New York, USA).
In order to obtain a representative sample of the N concentrations, two sub-samples per sample were analysed using a CNS analyser (Carlo Erba Instruments NA1500). The two sub-samples per sample were pooled for statistical analysis. NO_3^- and NH_4^+ concentrations in the soil pore water were measured colorimetrically using a Traacs 800+ auto-analyser. Soil pore water pH was measured with a Radiometer type PHM 82 pH meter.

Data analysis

All data were statistically analysed with the SPSS 11.5 package (SPSS Inc., Chicago, USA) after testing for normality. Data were log-transformed to correct for non-normality. Effects of N deposition on NH_4^+ and NO_3^- concentration and pH were tested with General Linear Model (GLM) procedures with repeated measures. Biomass, N content, chlorophyll concentrations and internal chemical composition of the plants were tested with one-way ANOVA with N treatment as fixed factor. Tukey's student range tests were used to identify differences between treatments.

RESULTS

Soil

During the two years of N addition, the concentrations of NO_3^- in the soil water showed a strong seasonal fluctuation (**figure 1**). NO_3^- concentrations were lower than 10 µmol l^{-1} at the start of the experiment. During the first winter period a significant increase of NO_3^- in the soil water was observed in the soils of the 40, 60 and 80 kg N ha^{-1} yr^{-1} treatments (**figure 1**; GLM, repeated measures, *d.f.* = 5, *F* = 37.95, *p* = 0.000). The NO_3^- concentration remained however very low at N additions of 1, 10 and 20 kg N ha^{-1} yr^{-1}. During spring the NO_3^- concentration rapidly decreased. In the second winter, an increase in NO_3^- (to ca. 550 µmol l^{-1}) could only be observed in the soil of the 80 kg N ha^{-1} yr^{-1} treatment. The NH_4^+ concentration remained constantly low (< 5 µmol l^{-1}) in all treatments (GLM, repeated measures, *d.f.* = 5, *F* = 2.26, *p* = 0.175, data not shown). No significant differences between the treatments were found for soil water pH either (GLM, repeated measures, *d.f.* = 5, *F* = 0.47, *p* = 0.788, data not shown). There was a clear positive correlation between the N input and the algal material of the upper soil layer (**figure 2**; GLM, *d.f.* = 5, *F* = 12.71, *p* = 0.004). Compared with the control situation (1 kg N ha^{-1} yr^{-1}), the soil of the 80 kg ha^{-1} yr^{-1} treatment contained three-fold more algae (**figure 2**). Algal material differed significantly between the treatment of 1 kg N ha^{-1} yr^{-1} and those with an N input of 40 kg N ha^{-1} yr^{-1} or higher.

Plant response

The above-ground and below-ground biomass of the grass species *C. epigejos* and *C. arenaria* significantly increased with increasing N deposition (**figures 3a, b; table 1**). After two years of N additions, the total biomass of the grasses (measured as above-ground and below-ground biomass) at a deposition rate of 80 kg N ha^{-1} yr^{-1} increased by 66% compared to the control treatment (data not shown). The total above-ground biomass of the grasses increased mainly as a result of an increase in biomass of *C. epigejos*, only a slight (but significant) increase was observed for the above-ground biomass of *C. arenaria* at an annual N deposition of 40 and 80 kg ha^{-1} (**table 1**). Below-ground biomass of both *C. epigejos* and *C. arenaria* increased significantly at deposition rates of 20 kg N ha^{-1} yr^{-1} and higher.

In the last year, the total biomass of both grass species together increased significantly compared to the biomass after two years in all treatments. *C. epigejos* biomass in the 80 kg N ha^{-1} yr^{-1} treatment nearly doubled in the third year. Although the biomass of *C. epigejos* increased significantly below-ground as well as above-ground, the biomass of *C. arenaria* did not (above-ground), or only slightly increase (below-ground) with increasing deposition rates (**table 1**). Moreover, total biomass of *C. arenaria* in the third year was equal or lower than the total biomass measured after two years. The shoot/root ratio of *C. epigejos* plants increased significantly from 1.5 in the lowest, to 2.3 in the highest N-addition treatment (**table 1**).

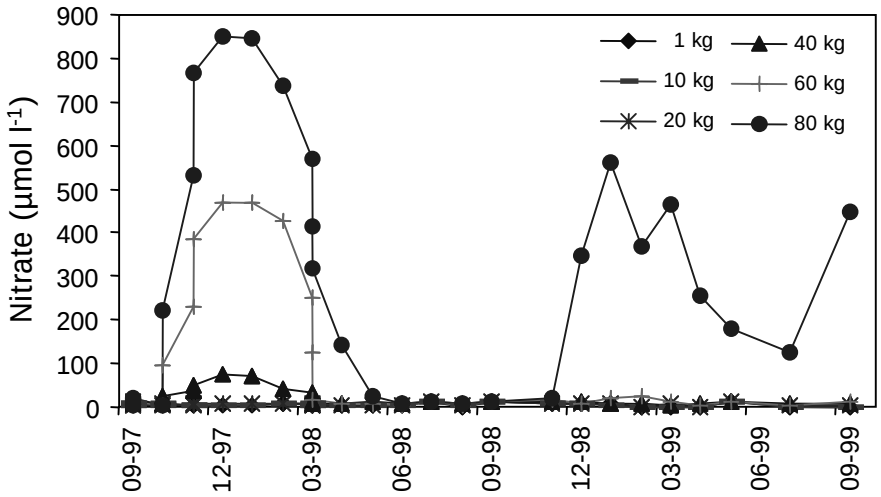

Figure 1: Nitrate concentration (µmol l^{-1}; *n* = 2) in soil pore water of the dune mesocosms at different nitrogen deposition rates, from September 1997 until September 1999.

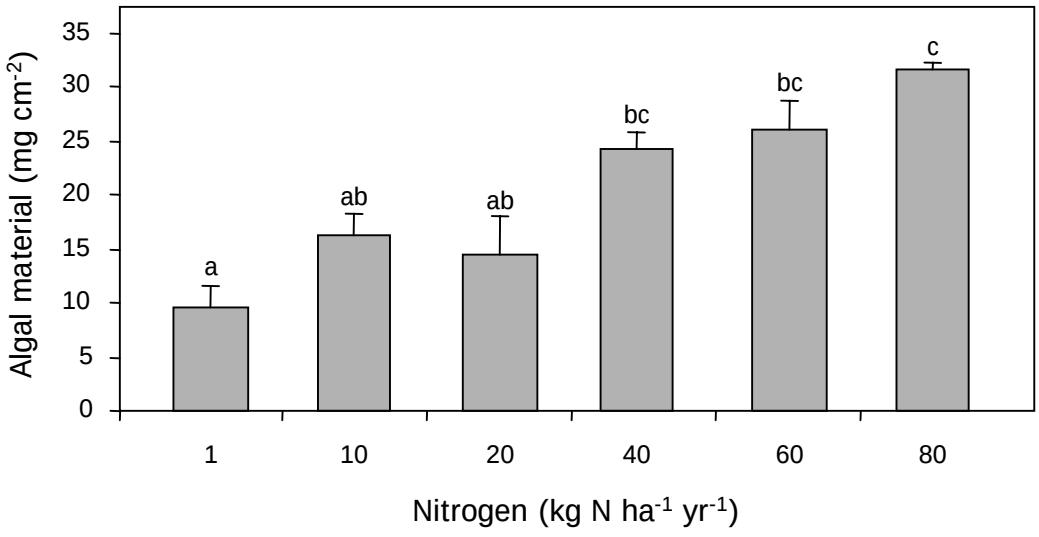

Figure 2: Algal material, measured as active chlorophyll concentration (mg cm^{-2}; means + s.e.; *n* = 4), in the top layer of the soil at different nitrogen deposition rates. Mean data of November 1997 and April 1998 are shown.

Biomass of *G. verum* and *Carlina vulgaris* did not differ between treatments after two years (**figure 4a; table 1**). After three years of N additions, all *Carlina vulgaris* plants had disappeared (data not shown) and both the below-ground and above-ground biomass of *G. verum* decreased with increasing N deposition (**figure 4b**). At N deposition rates above 20 kg ha^{-1} yr^{-1}, the decrease in total biomass (below-ground and above-ground) differed significantly from the control treatment (**figure 4b**).
The total amount of N stored in the vegetation was strongly elevated due to increased N deposition after two (GLM, *d.f.* = 5, F = 832.06, p = 0.000) and three (GLM, *d.f.* = 5, F = 401.61, p = 0.000) years of treatments. In the control situation ca. 14-15 kg N ha^{-1} was present in the above- and below-ground parts of the vegetation, whereas 94 kg ha^{-1} and 78 kg N ha^{-1} was stored in the vegetation in the highest N-addition treatment respectively after two and three years. 78-85% of the total amount of tissue N was stored in the above-ground biomass (data not shown).
N concentrations of the below-ground material of *C. epigejos* and *C. arenaria* and shoots of *C. arenaria* increased significantly after three years with increasing N deposition (**table 2**). P concentrations in the below-ground material of the tall grasses decreased with elevated N deposition. This was also visible in the shoots of *C. arenaria* (**table 2**). As a result, N/P ratios increased significantly in the below-ground material of both grasses and in the shoots of *C. arenaria*. In the shoots of *C. epigejos* trends of increasing N concentrations and decreasing P concentrations with increasing N deposition were visible, but were not significant. N/P ratios of both grass species were, apart from some sub-samples in the 1 and 10 kg N ha^{-1} yr^{-1} treatments, all above 16. The concentrations of the base cations Ca and Mn were significantly decreased in the above-ground material of *C. epigejos* and *C. arenaria* with increasing N deposition (**table 2**). Concentrations of Mg and K did not differ between treatments in above-ground and below-ground material. Chemical analysis on plant material (shoots) of *G. verum* was done in only four treatments (1-10-20-40 kg N ha^{-1} yr^{-1}) due to lack of material in the higher N deposition treatments. No significant differences could be detected for base cations. P concentrations increased significantly with increasing N deposition.

DISCUSSION

In this mesocosm experiment, the effects of N-deposition on a nutrient-poor, primary dune grassland were investigated. As a result of the artificial rain treatments with NH_4NO_3, the concentrations of NO_3^- in soil pore water changed during the experiment. In summer time, the NO_3^- concentration was low due to high uptake rates by the vegetation. During the first winter, an increase in NO_3^- was observed at deposition rates of 40, 60 and 80 kg N ha^{-1} yr^{-1}. At these rates, N input exceeded the N uptake by the vegetation. During the second winter, high NO_3^- concentrations were only measured at a deposition of 80 kg ha^{-1} yr^{-1}. Ongoing vegetation development in the third year clearly resulted in an increased NO_3^- uptake, decreasing NO_3^- concentrations in the soil pore water as compared to the concentrations measured in the second year. Indeed, above-ground and below-ground biomass of the grasses *C. epigejos* and *C. arenaria* increased with increasing N

deposition. An effect of elevated N fertilisation on the biomass of grasses was also found in several other studies (e.g. Boorman and Fuller, 1982; Bobbink *et al.*, 1988; Bobbink, 1991; Süß *et al.*, 2004). The increase in total above-ground biomass could almost completely be attributed to *C. epigejos*, whereas *C. arenaria* contributed most to the increase in total below-ground biomass. The high shoot/root ratios indicated that *C. epigejos* invested most of its energy and resources to above-ground biomass resulting in a high-competitive vigour for light.

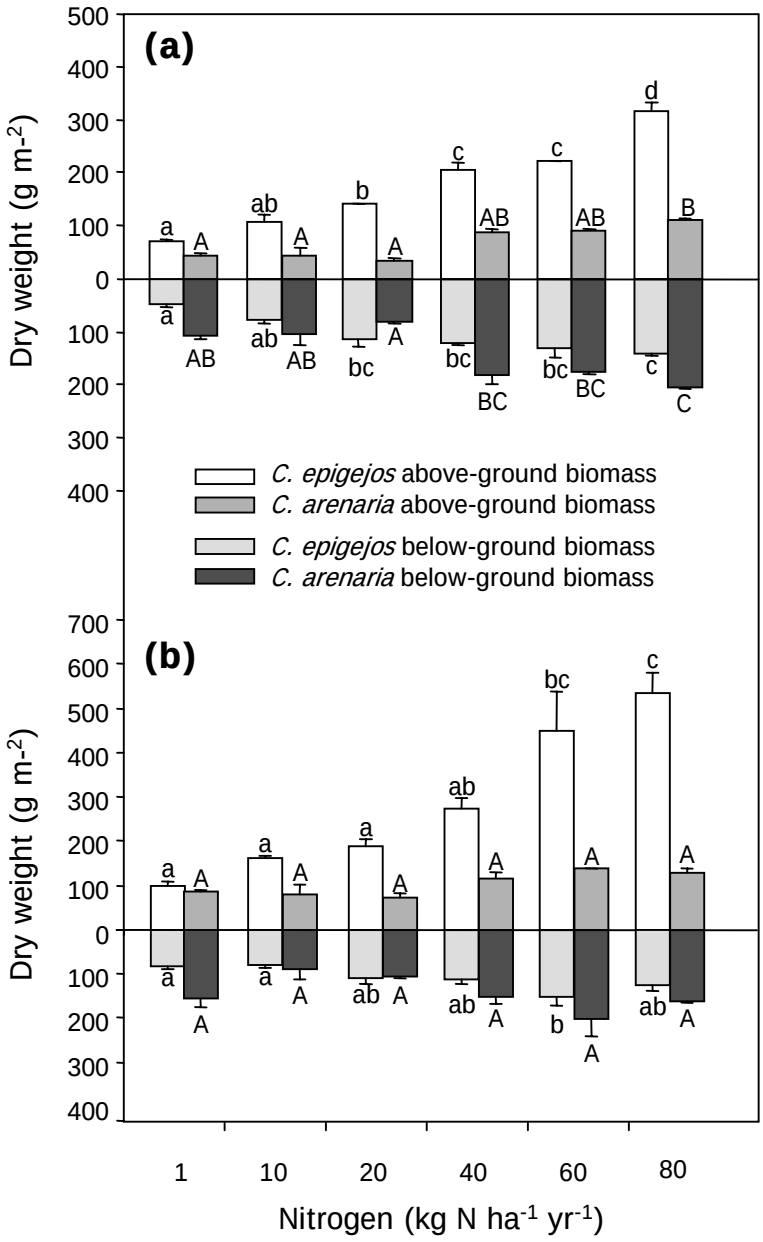

Figure 3: Above-ground biomass and below-ground biomass (g DW m^{-2}; means + s.e.) of *Calamagrostis epigejos* and *Carex arenaria* at different nitrogen deposition rates after two years **(a)** of N additions and after three years **(b)** of N additions. Statistical differences between treatments (Tukey-B test) are indicated by different letters.

C. arenaria responded differently; it tends to increase its tiller turnover rate by increasing rhizome length without increasing above-ground biomass, as was shown by lower shoot/root ratios. Total biomass did not increase with increasing N deposition and even showed a slight (but not significant) decrease after three years. This growth strategy is consistent with some more xeric sand dune species, which adapt to low rainfall by limiting above-ground biomass (and thus water requirements) and leaf area index (Ripley and Pammenter, 2004). Negative effects on biomass of the two grass species due to competition for light and/or nutrients were not measured. These were however not expected in a three year experiment, as both grasses showed different growth strategies, as described above.

Cation deficiency has been shown to occur in several plants species at high NH_4^+ concentrations (Marschner, 1995; De Graaf *et al.*, 2000). Our results show no indications of cation deficiency due to loss of base cations. This was due to low NH_4^+ concentrations in all treatments indicating high nitrification rates even in the first year after addition. Both grass species, but especially *C. arenaria* in shoots and roots, showed a significantly reduced P content and an increased N/P ratio as a result of N addition, indicating that both grass species were limited by P or tended towards P limitation (Koerselman and Meuleman 1996). As biomass increased with increasing N deposition, plants were also shown to be N limited. Apparently, the stress-tolerant competitors *C. arenaria* and *C. epigejos* are capable to increase their tiller turnover rate, or shoot length, even at low P concentrations. These results were in line with an earlier study showing high N/P ratios (29-35) in *Brachypodium pinnatum* after three years of N fertilisation in a calcareous grassland (Bobbink, 1991). Bobbink (1991) also found that *B. pinnatum* was N limited, even at these high N/P ratios and suggested that this grass was able to benefit more from N enrichment compared to other species (forbs) due to its ability to withstand very low concentrations P and its symbiotic growth with arbuscular mycorrhiza (AM; Bobbink, 1998).

The herbaceous species *G. verum* and *Carlina vulgaris* showed a strong decrease in total biomass in time, especially at elevated N loads (although not significant for *Carlina vulgaris*). Since *Carlina vulgaris* is a short-lived species, this species disappeared in all mesocosms, including the control treatment, after three years. Germination of new individuals was not found. Relatively high base cations concentrations were measured in above-ground biomass of *G. verum* at elevated N concentrations. In addition, P concentrations were relatively high and in a range which represented N limitation rather than P limitation (Koerselman and Meuleman, 1996). Therefore, competition for light with the graminoid species must be the driving process of the decrease and decline of *G. verum*, rather than toxicological or deficiency effects. It was shown that many grasses have a relatively higher growth rate compared to herbaceous species at elevated N concentrations and are better competitors for nutrients (Heil and Diemont, 1983; Berendse and Aerts, 1984; Roelofs, 1986; Aerts *et al.*, 1990). In addition, high coverage by these grasses results in reduced light penetration through the canopy, which hampers germination and growth of many short-lived or subordinate species (Bobbink, 1991; Ollf *et al.*, 1993). As a result, many herbaceous species may decline and plant biodiversity decreases.

Next to the effects on vegetation development, N enrichment has important implications for the top soil layer as it resulted in an increased growth of algal material. In dune grasslands and shifting dunes, this strongly prevents sand drift and the formation of blowouts due to wind action (see Hesp, 2002 and references therein). Blowouts are important for renewed vegetation succession and biodiversity as they allow occurrence of different successive stages. A correlation between N deposition and the amount of algae was also found in Finland by Poikolainen and others. (1998), who found an increase in the abundance of green algae on conifers at N deposition levels above 5-10 kg ha^{-1} yr^{-1}.

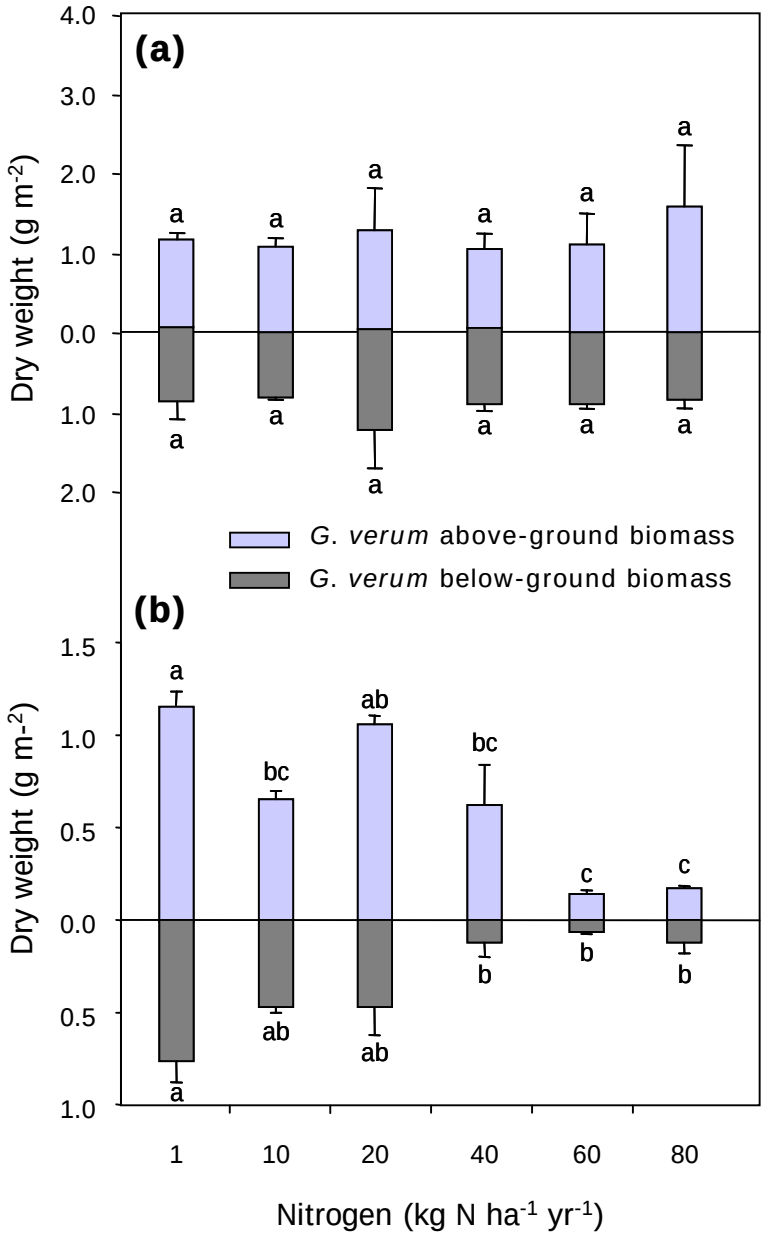

Figure 4: Above-ground biomass and below-ground biomass (g DW m^{-2}; means + s.e.) of *Galium verum* at different nitrogen deposition rates after two years **(a)** of N additions and after three years **(b)** of N additions. Statistical differences between treatments (Tukey-B tests) are indicated by different letters.

Table 1: Statistical results of the effect of nitrogen deposition on biomass and shoot/root ratio of *Galium verum*, *Carlina vulgaris*, *Carex arenaria* and *Calamagrostis epigejos* after 2 years and after 3 years. Degrees of freedom (*d.f.*), *F*-ratios and *p*-values are shown.

	Shoot			Root			Shoot/root		
Source	*d.f.*	*F*	*p*	*d.f.*	*F*	*p*	*d.f.*	*F*	*p*
Galium verum									
N deposition 2 years	5	0.26	0.918	5	0.52	0.754	5	0.92	0.529
N deposition 3 years	5	18.54	**0.001**	5	9.51	**0.008**	5	2.84	0.118
Carlina vulgaris									
N deposition 2 years	5	0.81	0.580	5	0.77	0.604	5	0.51	0.764
Carex arenaria									
N deposition 2 years	5	7.09	**0.017**	5	10.27	**0.007**	5	4.12	0.057
N deposition 3 years	5	5.44	**0.031**	5	3.70	0.071	5	1.51	0.313
Calamagrostis epigejos									
N deposition 2 years	5	82.25	**0.000**	5	10.99	**0.006**	5	6.27	**0.022**
N deposition 3 years	5	15.26	**0.002**	5	5.08	**0.036**	5	19.91	**0.001**
Total grass biomass (Carex arenaria and Calamagrostis epigejos)									
N deposition 2 years	5	87.00	**0.000**	5	23.86	**0.001**	5	0.120	0.983
N deposition 3 years	5	21.90	**0.001**	5	5.15	**0.035**	5	0.342	0.870

Table 2: Mean concentrations (µmol g^{-1} DW) and N/P ratios of the roots (**a**) and shoots (**b**) of *Galium verum, Carex arenaria* and *Calamagrostis epigejos*. Levels of significance are indicated as follows: *na* = not available, *ns* = not significant, * = $p < 0.01$, ** = $p < 0.05$, *** = $p < 0.001$.

(a)	*Calamagrostis epigejos* kg N ha^{-1} yr^{-1}							*Carex arenaria* kg N ha^{-1} yr^{-1}							*Galium verum* kg N ha^{-1} yr^{-1}						
Root	1	10	20	40	60	80	Sign.	1	10	20	40	60	80	Sign.	1	10	20	40	60	80	Sign.
Ca	201	155	171	144	118	128	*ns*	129	141	158	144	138	137	*ns*	372	368	356	316	*na*	*na*	*ns*
Mg	28	24	27	22	15	24	*ns*	25	26	23	20	19	19	*ns*	44	48	45	42	*na*	*na*	*ns*
K	74	74	90	83	43	74	*ns*	186	219	162	140	142	165	*ns*	181	223	230	154	*na*	*na*	*ns*
Mn	0.44	0.53	0.67	0.41	0.39	0.43	*ns*	0.63	0.50	0.41	0.49	0.37	0.25	*ns*	0.38	0.83	0.29	0.70	*na*	*na*	*ns*
P	14	13	14	11	7	9	***	12	9	8	7	7	6	**	33	31	38	28	*na*	*na*	*ns*
N	285	397	277	474	662	637	**	184	217	287	377	400	374	*	*na*	*na*	*na*	*na*	*na*	*na*	*na*
N/P	20	31	20	43	95	71	**	15	24	36	54	57	62	**	*na*	*na*	*na*	*na*	*na*	*na*	*na*

(b)	*Calamagrostis epigejos* kg N ha^{-1} yr^{-1}							*Carex arenaria* kg N ha^{-1} yr^{-1}							*Galium verum* kg N ha^{-1} yr^{-1}						
Shoot	1	10	20	40	60	80	Sign.	1	10	20	40	60	80	Sign.	1	10	20	40	60	80	Sign.
Ca	298	253	291	232	197	244	**	378	365	290	294	205	195	**	561	675	520	444	*na*	*na*	***
Mg	45	44	46	42	34	40	*ns*	23	27	25	37	31	29	*ns*	73	91	85	111	*na*	*na*	***
K	90	144	51	125	110	93	*ns*	166	140	150	123	116	102	*ns*	311	616	241	405	*na*	*na*	*ns*
Mn	1.01	1.14	1.42	0.71	0.57	0.66	**	3.77	4.26	2.86	1.96	1.53	0.89	**	0.47	0.74	0.45	0.23	*na*	*na*	***
P	19	18	14	13	10	10	*ns*	11	19	9	7	6	5	**	26	38	30	54	*na*	*na*	**
N	336	502	381	485	518	558	*ns*	284	352	283	545	432	523	**	*na*	*na*	*na*	*na*	*na*	*na*	*na*
N/P	18	28	27	37	52	56	*ns*	26	19	31	78	72	105	*	*na*	*na*	*na*	*na*	*na*	*na*	*na*

As a result of elevated N input, the total amount of N stored in the vegetation increased significantly. Considering a total N addition of only 4 kg ha^{-1} (including the dry deposition in the greenhouse) in the control treatment, most of the N taken up was obtained via mineralization. After two years of 10 kg N ha^{-1} yr^{-1} addition, the amount of N stored in the vegetation almost equals the amount of N supplied. Above 10 kg N ha^{-1} yr^{-1} the total amount of N stored in the vegetation is lower than the amount of N supplied. The vegetation in these treatments had taken up only a part of the added N whereas another part of the N left the system via drainage of NO_3^- or was taken up by algae. This is consistent with an earlier study, in which leaching of NO_3^- from N-fertilised soils was found at fertilisation rates of > 20 kg N ha^{-1} yr^{-1} (Ten Harkel and Van der Meulen, 1995). Since soil pore water was sampled in depth ranging from 0-15 cm, N concentrations are average values of the soil profile. Therefore soil fluxes could not be measured. Denitrification did not occur as anaerobic conditions were prevented successfully.

In our study, the first significant effects on vegetation development were found after two years of N addition (20 kg N ha^{-1} yr^{-1} and more), whereas most significant effects were found at deposition rates of 40 kg ha^{-1} yr^{-1} and higher. In contrast, others did not find an effect of N addition (25 kg N ha^{-1} yr^{-1}) on the vegetation composition of Dutch stable dune grassland after five years (Ten Harkel and Van der Meulen, 1995). However, Ten Harkel and Van der Meulen (1995) performed their N enrichment experiments at locations that already suffered from increased N loads (20-25 kg N ha^{-1} yr^{-1}) due to high N deposition. The effects of these N addition experiments may be very different from those measured with a low background deposition, as shifts in vegetation composition may occur at deposition rates lower than 20 kg N ha^{-1} yr^{-1}. In many dry dune grasslands in the U.K., for example, with an average N deposition of 10 kg ha^{-1} yr^{-1}, the vegetation is at present species-rich, whereas in many Dutch dune grasslands (average N load of 20-25 kg ha^{-1} yr^{-1}) grasses have become dominant (Kooijman and De Haan, 1995; Jones *et al.*, 2004).

Grass encroachment in dune grasslands in the U.K. was also found by Willis (1963), who demonstrated a strong increase in grasses such as *Festuca rubra*, *Poa pratensis* and *Agrostis stolonifera* upon N-fertilisation, indicating N limitation. In that study, it was also found that N (> 100 kg N ha^{-1} yr^{-1}) was more limiting plant growth than P and N enrichment resulted in a significant reduction of species numbers, especially annual species, lichens and mosses (Willis, 1963). Boorman and Fuller (1982) investigated the effects of experimental N addition on rabbit-grazed dune grasslands in the U.K. They also found a strong decline in annual species, lichens and mosses due to enhanced N treatments. A reduction in species number at elevated N deposition was also found by Jones and others (2003) at deposition rates of 20 kg N ha^{-1} yr^{-1}.

Studies by Kooijman *et al.* (1998) and Kooijman and Besse (2002) in dry dune grasslands along the Dutch coast revealed N limitation in non-calcareous dry dune ecosystems and co-limitation of N and P in calcareous dunes, based on plant and soil parameters. They concluded that atmospheric N deposition may cause grass encroachment in non-calcareous dunes, but probably only accelerates this process or succession in calcareous dune grasslands. In addition P is regarded to be an important limiting factor in many grasslands (e.g. Willis, 1963; Boorman and Fuller,

1982; Dougherty *et al.*, 1990; Ten Harkel and Van der Meulen, 1995). Our results are consistent with these findings and show a co-limitation of both P and N. It was also shown that in calcareous dune grasslands N loads above 20 kg ha^{-1} yr^{-1} cause changes in the vegetation cover, while loads higher than 40 kg ha^{-1} yr^{-1} can lead to dominance of grass species within a few years. This might have important implications for the field situations.
It was found that rabbit grazing prevented grasses (*F. rubra*) from becoming dominant at enhanced N input rates in English coastal dunes (Boorman and Fuller, 1982). In addition, many other studies revealed positive effects (higher species diversity, lower dominance of grasses) of (rabbit) grazing on the vegetation composition of dune grasslands (Ranwell, 1960; Kooijman and De Haan, 1995; Ten Harkel and Van der Meulen, 1995; Jutila, 2002). In a dune grassland in the Netherlands (with high annual N deposition rates, the cover of tall-grass communities increased and the cover of open communities decreased drastically in the ungrazed area, compared to the grazed area (Kooijman and De Haan, 1995). Grazing by rabbits seems essential to prevent graminoids to become dominant in the dry dunes, when N input rates are high (Ten Harkel and Van der Meulen, 1995). It is clear that rabbit grazing can generally not prevent grasses from becoming dominant given the high occurrence of dune grasslands dominated by tall grasses in the Netherlands at high levels of N deposition. Moreover, as grazing declines, either by reduced numbers of rabbits (rabbit populations fluctuate regularly), or abandonment of traditional management, many of the stable coastal dunes in the Netherlands are under serious threat of grass encroachment with ongoing atmospheric deposition.
Experiments as presented in this paper are necessary to establish empirical critical N loads for (semi-) natural ecosystems. The increased background levels in several European countries make it difficult to base these loads on field observations/ experiments. Therefore, experiments under controlled conditions in the laboratory or greenhouse, or in clean (low background deposition) environments, are of vital importance. Since vegetation development (grass encroachment and algal material increase) was found after two and three years at deposition levels of 20-40 kg ha^{-1} yr^{-1} and above, we argue that the empirical critical N load should be lower than 20-40 kg ha^{-1} yr^{-1} as this value defines a load, which results in a shift in vegetation development and composition on the long term. Although growth of the grass species seemed to stabilize after three years, the decline of *G. verum* would continue when we continued the experiment as a result of this grass encroachment. Considering the significant increase of *C. epigejos* at N deposition levels above 20 kg ha^{-1} yr^{-1} in the first two years, the critical N load for ungrazed calcareous dune grasslands will be around 10-20 kg N ha^{-1} yr^{-1} and will most likely tend to 10 kg N ha^{-1} yr^{-1}, as this is set to be a long-term critical load.

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REFERENCES

Aerts, R., Berendse, F., De Caluwe, H., Schmitz, M., 1990. Competition in heathland along an experimental gradient of nutrient availability. *Oikos*, 57: 310-318.

Arens, B., Jorritsma, J., Nieuwenhuizen, F. 2000. Schoorl: 100 jaar omgaan met dynamiek. *Aarde en Mens*, 3: 28-32.

Asman, W.A.H., Sutton, M.A., Schørring, J.K. 1998. Ammonia: Emission, atmospheric transport and deposition. *New Phytologist*, 139: 27-48.

Berendse, F., Aerts, R. 1984. Competition between *Erica tetralix* L. and *Molinia caerulea* (L.) Moench. as affected by the availability of nutrients. *Acta Oecologia/Oecologia Plantarum*, 5: 3-14.

Berlin, G.A.I., Linusson, A.C., Olsson, E.G.A. 2000. Vegetation changes in semi-natural meadows with unchanged management in southern Sweden, 1965-1990. *Acta Oecologica-International Journal of Ecology*, 21: 125-138.

Bobbink, R. 1998. Impacts of tropospheric ozone and airborne nitrogenous pollutants on natural and semi-natural ecosystems: a commentary. *New Phytologist*, 139: 161-168.

Bobbink, R. 1991. Effects of nutrient enrichment in Dutch chalk grassland. *Journal of Applied Ecology*, 28: 28-41.

Bobbink, R., Ashmore, M., Braun, S., Flückiger, W., Van den Wyngaert, I.J.J. 2003. Empirical nitrogen critical loads for natural and semi-natural ecosystems: 2002 update. In: Achermann, B., Bobbink, R. (Eds.). *Empirical critical loads for nitrogen - Proceedings. SAEFL*, Berne, Switzerland, pp. 43-171.

Bobbink, R., Bik, L., Willems, J.H. 1988. Effects of nitrogen fertilization on vegetation structure and dominance of *Brachypodium pinnatum* (L.) Beauv. in chalk grassland. *Acta Botanica Neerlandica*, 37: 231-242.

Bobbink, R., Heil, G.W. 1993. Atmospheric deposition of sulphur and nitrogen in heathland ecosystems. In: R. Aerts, G.W. Heil (Eds.). *Heathlands: Patterns and Processes in a changing environment. Geobotany 20.* Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 25-50.

Bobbink, R., Hornung, M., Roelofs J.G.M. 1996. Empirical nitrogen critical loads for natural and semi-natural ecosystems. In: *Manual on methodologies and criteria for mapping critical levels/loads and geographical areas where they exceeded*. UNECE Convention on Long-range Transboundary Air Pollution, Federal Environmental Agency, Berlin, Germany.

Bobbink, R., Hornung, M., Roelofs, J.G.M. 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, 86: 717-738.

Bobbink, R., Roelofs, J.G.M. 1995. Nitrogen critical loads for natural and semi-natural ecosystems: the empirical approach. *Water, Air and Soil Pollution*, 85: 2413-2418.

Bobbink, R., Willems, J.H. 1987. Increased dominance of *Brachypodium pinnatum* (L.) Beauv. in chalk grasslands: A threat to a species-rich ecosystem. *Biological Conservation*, 40: 301-314.

Boorman, L.A., Fuller, R.M. 1982. Effects of added nutrients on dune swards grazed by rabbits. *Journal of Ecology*, 70: 345-355.

Carroll, J.A Caporn, S.J.M., Johnson, D., Morecroft, M.D., Lee, J.A. 2003. The interactions between plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition. *Environmental Pollution*, 121: 363-376.

De Graaf, M.C.C., Bobbink, R., Verhagen, R.C.M., Crins, E., Roelofs, J.G.M. 2000. Effects of nitrogen nutrition and aluminium on seedling development of *Arnica montana* L. and *Cirsium dissectum* (L.) Hill. In: De Graaf, M.C.C. *Exploring the Calcicole-Calcifuge gradient in heathlands.* PhD Thesis. University of Nijmegen, Nijmegen, The Netherlands.

Dougherty, K.M., Mendelssohn, I.A., Monteferrante, F.J. 1990. Effect of nitrogen, phosphorous and potassium additions on plant biomass and soil nutrient content of a swale barrier strand community in Louisiana. *Annals of Botany*, 66: 265-271.

Erisman, J.W. 1993. Acid deposition to nature areas in the Netherlands: part I. Methods and results. *Water, Air and Soil Pollution*, 71: 51-80.

Galloway, J.N., Cowling, E.B. 2002. Reactive nitrogen and the world: 200 years of change. *Ambio*, 16: 64-71.

Galloway, J.N. 2000. Nitrogen mobilization in Asia. *Nutrient Cycling in Agroecosystems*, 57: 1-12.

Harriman, R., Curtis, C., Edwards, A.C. 1998. An Empirical approach for assessing the relationship between nitrogen deposition and nitrate leaching from upland catchments in the United Kingdom using runoff chemistry. *Water, Air and Soil Pollution*, 105: 193-203.

Heil, G.W., Diemont, W.H. 1983. Raised nutrient levels change heathland into grassland. *Vegetatio*, 53: 113-120.

Hesp, P. 2002. Foredunes and blowouts: initiation, geomorphology and dynamics. *Geomorphology*, 48: 245-268.

Jones, M.L.M. Hayes, F., Brittain, S.A. Haria, S., Williams, P.D., Ashenden, T.W., Norris, A.D. Reynolds, B. 2003. Field survey linking soil, vegetation and groundwater parameters in UK sand dunes with atmospheric nitrogen deposition. In: Achermann, B., Bobbink, R. (Eds.). *Empirical critical loads for nitrogen - Proceedings.* SAEFL, pp. 291-293.

Jones, M.L.M., Wallace, H.L., Norris, D., F., Brittain, S.A. Haria, S., Jones, R.E., Rhind, P.M., Reynolds, B., Emmett, B.A. 2004. Changes in vegetation and soil characteristics in coastal sand dunes along a gradient of atmospheric nitrogen deposition. *Plant Biology*, 6(5): 598-605.

Jutila, H. 2002. How does grazing by cattle modify the vegetation of coastal grasslands along the Baltic Sea? *Annales Botanici Fennici*, 38(3): 181-200.

Klapwijk, G.J. 1996. Een schets van het duinbeheer tot de 18e eeuw. *Meijendel Med.*, 30: 1-12.

Koerselman, W., Meuleman, A.F.M. 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology*, 33: 1441-1450.

Kooijman, A.M., Besse, M. 2002. The higher availability of N and P in lime-poor than ion lime-rich coastal dunes in the Netherlands. *Journal of Ecology*, 90: 394-403.

Kooijman, A.M., De Haan, M.W.A. 1995. Grazing as a measure against grass encroachment in Dutch dry dune grassland: effects on vegetation and soil. *Journal of Coastal Conservation*, 1: 127-134.

Kooijman, A.M., Dopheide, J.C.R., Sevink, J., Takken I., Verstraten, J.M. 1998. Nutrient limitations and their implications on the effects of atmospheric deposition in coastal dunes; lime-poor and lime-rich sites in the Netherlands. *Journal of Ecology*, 86: 511-526.

Kooijman, A.M., Van der Meulen, F. 1996. Grazing as a control against 'grass-encroachment' in dry dune grasslands in the Netherlands. *Landscape Urban Planning*, 34: 323-333.

Krupa, S.V. 2003. Effects of atmospheric ammonia (NH_3) on terrestrial vegetation: a review. *Environmental Pollution*, 124: 179-221.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants* (2nd edition). Academic Press, London, United Kingdom.

Morecroft, M.D., Sellers, E.K., Lee, J.A. 1994. An experimental investigation into the effects of atmospheric nitrogen deposition on two semi-natural grasslands. *Journal of Ecology*, 82: 475-483.

Ollf, H., Huisman, J., Van Tooren, B.F. 1993. Species dynamics and nutrient accumulation during early primary succession in coastal sand dunes. *Journal of Ecology*, 81: 693-706.

Poikolainen, J., Lippo, H., Hongisto, M., Kubin, E., Mikkola, K., Lindgren, M. 1998. On the abundance of epiphytic green algae in relation to the nitrogen concentrations of biomonitors and nitrogen deposition in Finland. *Environmental Pollution*, 102: 85-92.

Ranwell, D.S. 1960. Newborough Warren, Anlesey. III. Changes in the vegetation on parts of the dune system after loss of rabbits by myxomatosis. *Journal of Ecology*, 48: 385-397.

Ripley, B.S., Pammenter, N. W. 2004. Do low standing biomass and leaf area index of sub-tropical coastal dunes ensure that plants have an adequate supply of water? *Oecologia*, 139: 535-544.

Roelofs, J.G.M. 1986. The effect of airborne sulphur and nitrogen deposition on aquatic and terrestrial heathland vegetation. *Experientia*, 42: 372-377.

Süß, K., Storm, C., Zehm, A., Schwabe, A. 2004. Succession in inland sand ecosystems: which factors determine the occurrence of the tall grass species *Calamagrostis epigejos* (L.) Roth and *Stipa capillata* L.? *Plant Biology*, 6(4): 465-476.

Ten Harkel, M.J., Van der Meulen, F. 1995. Impact of grazing and atmospheric nitrogen deposition on the vegetation of dry coastal dune grasslands. *Journal of Vegetation Science*, 6: 445-452.

Veer, M.A.C., Kooijman, A.M. 1997. Effects of grass-encroachment on vegetation and soil in Dutch dry dune grasslands. *Plant and Soil*, 192: 119-128.

Williams, O.B., Wells, T.C.E., Wells, D.A. 1974. Grazing management of Woodwalton fen: seasonal changes in the diet of cattle and rabbits. *Journal of Applied Ecology*, 11: 499-516.

Willis, A.J. 1963. Braunton Burrows: the effects on the vegetation of the addition of mineral nutrients to the dune soils. *Journal of Ecology*, 51: 353-374.


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Chapter 3

DECLINE OF ACID-SENSITIVE PLANT SPECIES IN HEATHLAND CAN BE ATTRIBUTED TO AMMONIUM TOXICITY IN COMBINATION WITH LOW PH

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SUMMARY The effects of increasing ammonium concentrations in combination with different pH levels were studied on five heathland plant species, to determine whether their occurrence and decline could be attributed to ammonium toxicity and/or pH levels. Plants were grown in growth media amended with four different ammonium concentrations (10, 100, 500 and 1000 μmol l^{-1}) and two pH levels, resembling acidified (pH 3.5 or pH 4) and weakly buffered (pH 5 or pH 5.5) situations, respectively. Survival of *Antennaria dioica* and *Succisa pratensis* was reduced by low pH in combination with high ammonium concentrations. Biomass decreased with increased ammonium concentrations and decreasing pH levels. Internal pH of the plants decreased with increasing ammonium concentrations. Survival of *Calluna vulgaris*, *Deschampsia flexuosa* and *Gentiana pneumonanthe* was not affected by ammonium. Moreover, biomass increased with increasing ammonium concentrations. Biomass production of *G. pneumonanthe* reduced at low pH levels. A decline of acid-sensitive species in heathlands was attributed to ammonium toxicity effects in combination with a low pH.

INTRODUCTION

In Western Europe, heathlands and matgrass swards were once common vegetation types (De Smidt, 1975; Gimmingham, 1992). From the mid-eighteenth century, however, European heathlands declined dramatically in area, mainly as a result of reclamation for forestry and farming (De Smidt, 1975, 1979; Webb, 2002) and their unique composition of many characteristic and endangered plant and animal species makes these ecosystems subject to conservation and restoration (Gimingham, 1992; Webb, 1998). In addition to losses through habitat destruction, the biodiversity of dry and wet heathlands and related matgrass swards is rapidly declining (Roelofs *et al.*, 1996; Bobbink *et al.*, 1998). Plant species diversity in heathlands in the Netherlands has declined over 50% in the last fifty years due to environmental stress (Vonk *et al.*, 2001) and many herbaceous species such as *Antennaria dioica*, *Arnica montana*, *Cirsium dissectum* and *Gentiana pneumonanthe* have largely disappeared and are seriously threatened. Conversely, grasses such as *Deschampsia flexuosa* and *Molinia caerulea* have become dominant.

It was suggested that this shift towards grass-dominated vegetation was caused by the increased atmospheric deposition of nitrogen (N) and sulphur (S)-containing compounds during the last decades, resulting in eutrophication and acidification of the soil (Heil and Diemont, 1983; Bobbink *et al.*, 1998). Earlier studies showed that grasses had a higher productivity at elevated N concentrations compared to herbaceous species and shrubs (Heil and Diemont, 1983; Berendse and Aerts, 1984; Roelofs, 1986; Aerts *et al.*, 1990). Grasses were therefore thought to out-compete herbaceous species and shrubs at high N availability (Aerts and Berendse, 1988; Pitcairn *et al.*, 1991). However, a study by Houdijk *et al.* (1993) showed that many herbaceous species already disappeared from the heathlands before grasses became dominant, indicating that processes other than competition for light and nutrients play an important role in the decline of herbaceous species.

Nitrogen deposition in the Netherlands is mainly in the form of ammonium (NH_4^+; Erisman, 1990; Bobbink *et al.*, 1992; Boxman *et al.*, 1998; Kreutzer *et al.*, 1998) and, as a result, plants encounter increased NH_4^+ concentrations, which have been shown to be toxic for many plant species (e.g. Mehrer and Mohr, 1989; Britto and Kronzucker, 2002, and references herein), including herbaceous heathland species like *A. montana* and *C. dissectum* (De Graaf *et al.*, 1998; Dorland *et al.*, 2003; Lucassen *et al.*, 2003). In addition, the atmospheric deposition of S and N results in acidification of heathland soils and the depletion of buffering base cations such as Ca^{2+}, Mg^{2+} and K^+ (Carnol *et al.*, 1997). This may lead to a change in soil buffering and the loss of acid-sensitive species (Houdijk *et al.*, 1993). For degraded acidified dry heathlands an average soil pH of 3.8-4.2 was found, whereas for degraded acidified wet heaths a slightly higher pH of 4.0-4.5 (due to reduction processes) was measured (De Graaf *et al.*, 1994; Roelofs *et al.*, 1996; Dorland *et al.*, 2003). Acidification in wet heaths is also induced by lowering of groundwater tables as this enhances acidifying processes such as the oxidation of iron sulphides, mineralization and influence of acidic rain water (Roelofs, 1993; Runhaar *et al.*, 1996; Grootjans *et al.*, 1996; Lamers *et al.*, 1998). Due to acid soil conditions, nitrification in heathland soils is low and is strongly reduced with decreasing pH (Roelofs *et al.*, 1985; Van Breemen and Van Dijk, 1988; Dorland *et*

al., 2004). Since nitrification is an acidifying process, the inhibition of nitrification constitutes a negative feedback on acidification. All these processes contribute directly or indirectly to an accumulation of NH_4^+ in the soil, mostly accompanied by higher NH_4^+ availabilities.

Although the external pH was suggested as the primary cause for the decline of herbaceous species in favour of grasses (Van Dam *et al.*, 1986; Dueck and Elderson, 1992; Houdijk *et al.*, 1993), others found no effects of external pH on the growth of herbaceous heathland species (Van Dobben, 1991), or found indirect pH effects through aluminium toxicity (Heijne *et al.*, 1996). NH_4^+ concentrations did not explain the dramatic decline in plant diversity either, since many herbaceous species where shown to grow well on high NH_4^+ concentrations at weakly buffered conditions (Bobbink *et al.*, 2003).

The relationship between NH_4^+ toxicity and soil acidification has been the subject of a number of studies (Findenegg, 1987; Dijk and Eck, 1995; Dijk and Grootjans, 1998; Lucassen *et al.*, 2003). Lucassen *et al.* (2003) suggested that the decline of *C. dissectum* was due to the combination of high NH_4^+ concentrations and a low pH of the growth medium. They hypothesized that at high NH_4^+ concentrations and low external pH, *C. dissectum* suffered from low internal pH levels as a result of reduced proton excretion. This is explained by proton excretion, which was found to occur when NH_4^+ is assimilated in the plants (Raven and Smith, 1976; Findenegg, 1987; Van Beusichem *et al.*, 1988; Goodchild and Givan, 1990). Others also found that growth on NH_4^+ results in a decrease in tissue pH (Gerendás *et al.*, 1990, De Graaf *et al.*, 2000).

In this study we describe the results of a hydroponic experiment with five heathland species from both wet and dry heaths: *G. pneumonanthe*, *Succisa pratensis*, *Calluna vulgaris*, *A. dioica* and *D. flexuosa*. Plants were subjected to environmental stress by growing them in media with different NH_4^+ concentrations and pH levels. Biomass, mortality, internal pH of the plants and the chemical composition of the plants were measured to estimate fitness and survival of the plants. NH_4^+-tolerant species are usually found in acidic habitats and are likely to be adapted to NH_4^+ nutrition, since NH_4^+ is the dominant N form at low pH (Gigon and Rorison, 1972; Troelstra *et al.*, 1990). In contrast, species from less acidic habitats usually prefer nitrate (Falkengren-Grerup and Lakkenborg-Kristensen, 1994; Britto and Kronzucker, 2002). Therefore, we hypothesized that acidification would negatively affect the survival and fitness of *A. dioica* and *S. pratensis*, as these are characteristic for weakly buffered conditions and regarded as acid-sensitive species. It was also expected that these effects will be enhanced by increased NH_4^+ concentrations. *Calluna vulgaris* and *D. flexuosa* can be found in eutrophied and acidified heathlands and are therefore thought to be more acid-tolerant. These species were expected to be less affected by low external pH in combination with high NH_4^+ concentrations. The rare herbaceous species *G. pneumonanthe* is regarded as slightly acid-tolerant and thus less susceptible to low external pH than *A. dioica* and *S. pratensis*.

MATERIALS AND METHODS

Plant species

A. dioica (L.) Gaertner, *G. pneumonanthe* (L.) and *S. pratensis* (L.) Moench are perennial herbaceous species characteristic for species-rich, nutrient-poor matgrass swards and heathlands. In the Netherlands, *A. dioica* can be found on the dry parts of nutrient-poor heathlands and species-rich grasslands. *G. pneumonanthe* is mainly found on wet heaths and species-rich grasslands. Both species are rare and threatened and their present occurrence in the Netherlands is restricted to a few areas. *S. pratensis* is a more widespread species, found on various types of nutrient-poor grasslands such as matgrass swards and species-rich heathlands. Its distribution has been reduced by almost 75% since 1935 and it is listed on the red list since 2002 (Van der Meijden, 2002). *Calluna vulgaris* Hill is a common shrub, characteristic for dry heaths and often dominating in species-poor heaths. Its distribution is widespread in Western Europe. In deteriorating heathlands, suffering from acidification and eutrophication, *Calluna vulgaris* is replaced by grasses such as *D. flexuosa* (L.) Trin. *D. flexuosa* is a common grass, which mainly occurs in N-enriched matgrass swards and heathlands, where it can reach cover percentages up to 100%.

Table 1: NH_4^+ amendments (in µmol l^{-1}) and pH ranges for each species.

	pH range		
	Low pH	High pH	NH_4^+ concentration (µmol l^{-1})
Gentiana pneumonanthe	4	5.5	10 - 100 - 500 - 1000
Succisa pratensis	4	5.5	10 - 100 - 500 - 1000
Antennaria dioica	3.5	5	10 - 100 - 500 - 1000
Calluna vulgaris	3.5	5	10 - 100 - 500 - 1000
Deschampsia flexuosa	3.5	5	10 - 100 - 500 - 1000

Experimental design

Seedlings of *A. dioica*, *G. pneumonanthe* and *S. pratensis* were obtained by germinating seeds on nutrient-poor substrate (vermiculite). The seedlings were grown for 3 to 5 weeks prior to the start of the experiments. Seeds of *G. pneumonanthe* and *S. pratensis* were collected in a Dutch nature reserve (Havelte-Oost; 52° 48'N, 6° 13'E), *A. dioica* seeds were ordered from Blauetikett-Bornträger GmbH, Offstein, Germany. Seedlings of *Calluna vulgaris* and *D. flexuosa* were collected in a heathland near Nijmegen (Mulderskop; 51°47'N, 5°53'E) and grown on vermiculite for 2 weeks prior to the start of the experiment. In order to reduce initial differences between plants, plants of approximately the same size were selected.

Plants were carefully placed in polystyrene trays and transferred into 2-l opaque containers, which were continuously aerated to prevent anoxic conditions. The seedlings were exposed to eight different growing solutions differing in NH_4^+ concentration and pH level. The NH_4^+ concentrations were 10, 100, 500 and 1000 µmol l^{-1}. The pH levels resembled that of acidified and weakly buffered soil

conditions of dry and wet heaths, respectively. Therefore, *G. pneumonanthe* and *S. pratensis* were subjected to pH values of 4 (acidified) and 5.5 (weakly buffered), whereas *A. dioica*, *Calluna vulgaris* and *D. flexuosa* were subjected to pH values of 3.5 (acidified) and 5 (weakly buffered; **table 1**). The number of plants in each container was: three for *S. pratensis*, four for *G. pneumonanthe*, seven for *D. flexuosa* and *A. dioica and* eight for *Calluna vulgaris.*

We added low amounts of nutrients to the culture media in order to represent natural conditions according to De Graaf *et al.* (1994). The concentrations of nutrients were 100 µmol l^{-1} Ca^{2+}, 100 µmol l^{-1} Mg^{2+}, 200 µmol l^{-1} K^{+}, 100 µmol l^{-1} SO_4^{2-}, 200 µmol l^{-1} PO_4^{3-}, 0.27 µmol l^{-1} Fe, 0.7 µmol l^{-1} Zn^{2+}, 0.8 µmol l^{-1} Mn^{2+}, 0.2 µmol l^{-1} Cu^{2+}, 0.008 µmol l^{-1} Mo, 0.8 µmol l^{-1} H_3BO_3. Fe was added as Fe-EDTA. 1-cyanguanidine was added to the culture media in a concentration which was 1% of the molar concentration of NH_4^{+} to prevent nitrification. Nitrate concentrations were low throughout the experiment, indicating that nitrification was successfully reduced by the application of 1-cyanguanidine and the relative high flow velocity of medium through the trays. Growth media in the containers were continuously refreshed using a continuous flow system. Per container, 12.5-l medium was pumped weekly from 25 l reservoirs. The pH of the growth solutions was set using 1 mmol l^{-1} HCl and 1 mmol l^{-1} NaOH. The pH of the growth solutions was checked every two days and adjusted when necessary (data not shown). The experiments were performed in a climate chamber with a day/night regime of 16/8 hours and a temperature of 20 and 17 °C, respectively. The humidity was 50-70% and the irradiance was 200 µmol m^{-2} s^{-1}.

Survival and growth

During the experiment, mean survival per container was determined by measuring the percentage of plants that died weekly. *D. flexuosa*, *G. pneumonanthe* and *S. pratensis* were harvested after 8 weeks, whereas *A. dioica* was harvested after 6 weeks. The experimental period of *Calluna vulgaris* was 14 weeks, since this is a slow growing species and visible effects of treatments were expected to occur later. After harvesting, plants were pooled per container and separated into shoots and roots. Total dry weight (biomass) was determined after drying the plant material for 24 hours at 70°C.

Internal pH and nutrient composition

In order to measure internal pH of the plants, plants were pooled per container and mixed thoroughly. Then 0.1-0.2 g of the mixed plant material was ground with 1 or 2 ml demineralized water, respectively, in a glass-to-glass Potter homogenising device. A Sentron pH sensor in combination with a Sentron 1001 pH meter was used to measure pH in the Potter tube directly. From the dried plants of *G. pneumonanthe*, *Calluna vulgaris* and *D. flexuosa*, root and shoot material was sampled and ground using liquid N. Then, 100 mg of plant material was digested in sealed Teflon vessels in a Milestone destruction microwave oven (MLS 1200 mega) with nitric acid and hydrogen peroxide. Plant material was analysed for Ca, Mg, P, Mn and S using an inductively-coupled plasma emission spectrophotometer (ICP, Spectroflame Flame

VML2). K and Na were determined with a flame photometer, using a Technicon I Auto Analyser (Technicon, New York, USA). To obtain a representative sample of the carbon (C) and N concentrations, two sub-samples per sample were analysed using a Carlo Erba Na 1500 CNS analyser. The two sub-samples were pooled for statistical analysis. Variation among the sub samples was small; Standard deviations were at most 5% of the means for both carbon and nitrogen concentrations. The nutrient composition and C and N content of *A. dioica* and *S. pratensis* could not be analysed due to lack of material, which was caused by the high mortality and the small size of the plants.

Statistical analysis

All data were analysed using the SPSS 11.5 package (SPSS Inc., Chicago, USA) after testing for normality. Mean survival per container was analysed using a general linear model (GLM) procedure. Survival was monitored regularly, but only final survival data was included in the statistical analysis, since we were mainly interested in the effects of the treatments on total survival. Survival percentages were arcsin of square root transformed on the survival factor (percentage/100). The effects of NH_4^+ and external pH levels on shoot and root biomass, internal pH and internal chemical composition were analysed using a GLM procedure.
Due to the high mortality in some of the treatments, as opposed to low mortality (high survival) in other treatments, data on the species *A. dioica* and *S. pratensis* were unbalanced. Although mean biomass and mean internal pH are generally good measures for treatment effects, they were however not always good measures (as tested with GLM) in the treatments with very low survival. Results should be interpreted carefully considering the small number of plants the measures are based on.

RESULTS

Survival

Increased NH_4^+ concentrations resulted in decreased survival of *S. pratensis* and *A. dioica* (**figures 1a, b; table 2**). At the lowest NH_4^+ concentration (10 µmol l^{-1}) survival of *S. pratensis* and *A. dioica* ranged between 100 and 80%, while the survival of both species rapidly decreased at higher NH_4^+ concentrations to as low as 0% for *S. pratensis* at 500 µmol l^{-1}. Although increased NH_4^+ concentrations resulted in slightly reduced survival of *Calluna vulgaris* and *D. flexuosa*, no significant NH_4^+ effect was observed (**figures 1c, d; table 2**). The survival of *S. pratensis* and *A. dioica* was significantly affected by the pH of the growth medium at the $p < 0.001$ level (**figures 1a, b; table 2**). Survival of these species was lower at low pH (average survival percentage of 38 and 60 for *S. pratensis* and *A. dioica*, respectively) compared to the survival at high pH (average survival percentage of 65 and 91 for *S. pratensis* and *A. dioica* respectively). For both *S. pratensis* and *A. dioica*, an NH_4^+ * pH interaction effect was shown at the $p < 0.1$ level, suggesting enhanced detrimental effects of high NH_4^+ concentrations and low external pH. No significant external pH effects on survival of

Calluna vulgaris, D. flexuosa and *G. pneumonanthe* were observed. Although no significant effects of NH_4^+ or pH on survival of *Calluna vulgaris* were found, an interacting effect between NH_4^+ and pH on the survival was found at the $p < 0.1$ level (**figure 1c; table 2**). The effect of NH_4^+ and pH on survival of *G. pneumonanthe* was not tested, since no mortality was observed during the experiment (data not shown).

Table 2: Statistical results of the effects of different NH_4^+ and pH levels on survival of *Succisa pratensis*, *Antennaria dioica*, *Calluna vulgaris* and *Deschampsia flexuosa*.

Source	*d.f.*	*Mean Square*	*F*†
Succisa pratensis			
NH_4	3	2.43	36.80 ***
pH	1	1.49	22.50 ***
NH_4^+ * pH	3	0.19	2.92 +
error	24	0.07	
Antennaria dioica			
NH_4	3	0.35	21.53 ***
pH	1	1.81	113.05 ***
NH_4^+ * pH	3	0.05	2.98 +
error	24	0.02	
Calluna vulgaris			
NH_4	3	0.05	0.99
pH	1	0.00	0.03
NH_4^+ * pH	3	0.12	2.34 +
error	24	0.05	
Deschampsia flexuosa			
NH_4	3	0.03	0.60
pH	1	0.00	0.00
NH_4^+ * pH	3	0.04	0.77
error	24	0.05	

† Significance levels are indicated by: + $p < 0.10$, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Biomass

Both shoot and root biomass varied at different NH_4^+ concentrations and pH levels (**figures 2a-e; table 3**). *G. pneumonanthe* and *D. flexuosa* showed increased shoot biomass with increasing NH_4^+ concentrations at both pH levels. Root biomass of *G. pneumonanthe* and *D. flexuosa* did not significantly differ between different NH_4^+ levels. At pH 5, shoot and root biomass of *A. dioica* increased with increasing NH_4^+ levels from 10 up to 100 µmol l^{-1}, whereas higher NH_4^+ levels (500 and 1000 µmol l^{-1}) resulted in lower biomass. This indicated that *A. dioica* performed best at an NH_4^+ concentration of 100 µmol l^{-1}. Below this concentration *A. dioica* was limited by N and above 100 µmol l^{-1} biomass of shoots and roots was negatively affected by high NH_4^+

concentrations. Similar patterns showing optimal NH_4^+ concentrations for biomass production of the shoots were detected at both pH levels for *S. pratensis* and at pH 3.5 for *Calluna vulgaris*. At pH 5.5, NH_4^+ concentrations up to 500 µmol l^{-1} resulted in increased shoot biomass of *S. pratensis* (up to 0.2 g DW; **figure 2b; table 3**) and lower (0.01 g DW) shoot biomass was observed at 1000 µmol l^{-1} NH_4^+. At the low pH levels, the highest shoot biomass for *Calluna vulgaris* (0.2 g DW) was measured at 500 µmol l^{-1} NH_4^+ and at 100 µmol l^{-1} for *S. pratensis* (0.08 g DW). However, the biomass of *S. pratensis* of the plants that survived at pH 4 was low at 500 and 1000 µmol l^{-1} NH_4^+ (**figure 1a**) and results shown in **figure 2b** should therefore be interpreted carefully.
The external pH of the medium markedly affected the biomass of the shoots and roots of *G. pneumonanthe* and *A. dioica*. At low pH levels, shoot and root biomass were on average 44% and 80% lower for *G. pneumonanthe* and *A. dioica*, respectively, as compared to the biomass at the higher pH levels (**figures 2a, c**). A negative, but non-significant, trend between biomass production and decreasing pH was visible for *S. pratensis* (**figure 2b; table 3**). A significant interaction effect between NH_4^+ and pH of the growth medium on the root biomass was found for *A. dioica*, suggesting that negative pH effects were enhanced at high NH_4^+ concentrations.

Internal pH

Internal pH levels varied widely between the plant species and treatments, ranging from almost 6.5 to 4 (**figure 3**). The pH of the roots of *G. pneumonanthe* (average of pH 4.27) was significantly lower compared to the pH of the shoots (average of pH 5.04; One-way ANOVA, MS = 9.379, *d.f.* = 1, $F = 205.331$, $p < 0.001$, data not shown), whereas no significant differences between shoot and root pH of the other species were observed. In general, increasing NH_4^+ concentrations decreased internal pH levels significantly in both root and shoot material (**figure 3; table 4**). Only in the roots of *A. dioica* there was a (non-significant) trend of a slightly higher internal pH at high NH_4^+ concentrations in the pH 3.5 treatment (One-way ANOVA, *MS* = 0.151, *d.f.* = 3, $F = 0.810$, $p = 0.514$).
Acidity of the growth medium significantly influenced only the internal pH of shoots and roots of *A. dioica*. At an external pH of 3.5, the pH of shoots and roots was on average 4.7, whereas the pH of shoots and roots ranged between 5 and 6.5 when grown in a medium of pH 5 (average 5.9; **figure 3c**). Internal pH of shoots and roots of *S. pratensis* at 500 µmol l^{-1} NH_4^+ in the pH 5.5 treatment was low (4.5 and 4.3, respectively) and did not significantly differ from the pH levels at 1000 µmol l^{-1} NH_4^+ (4.8 and 4.7, respectively). Since all plants had died in the treatments with 500 µmol l^{-1} NH_4^+ at pH 4, there were no data for this treatment. Results of the internal pH of plants grown in 1000 µmol l^{-1} NH_4^+ at pH 4 should be interpreted carefully since these were based on a limited number of plants.
The pH of the external medium enhanced the effects of the NH_4^+ concentrations for *A. dioica* and *D. flexuosa*, illustrated by significant NH_4^+ * pH interaction effects (**table 4**). The internal pH of *A. dioica* and *D. flexuosa* showed a decrease at pH 5 with increasing NH_4^+ concentrations, whereas at pH 3.5 no significant NH_4^+ effects were measured (**figures 3c, e**). Thus, the effects of NH_4^+ on internal pH differed significantly between pH levels, which explains the interaction effect in **table 4**.

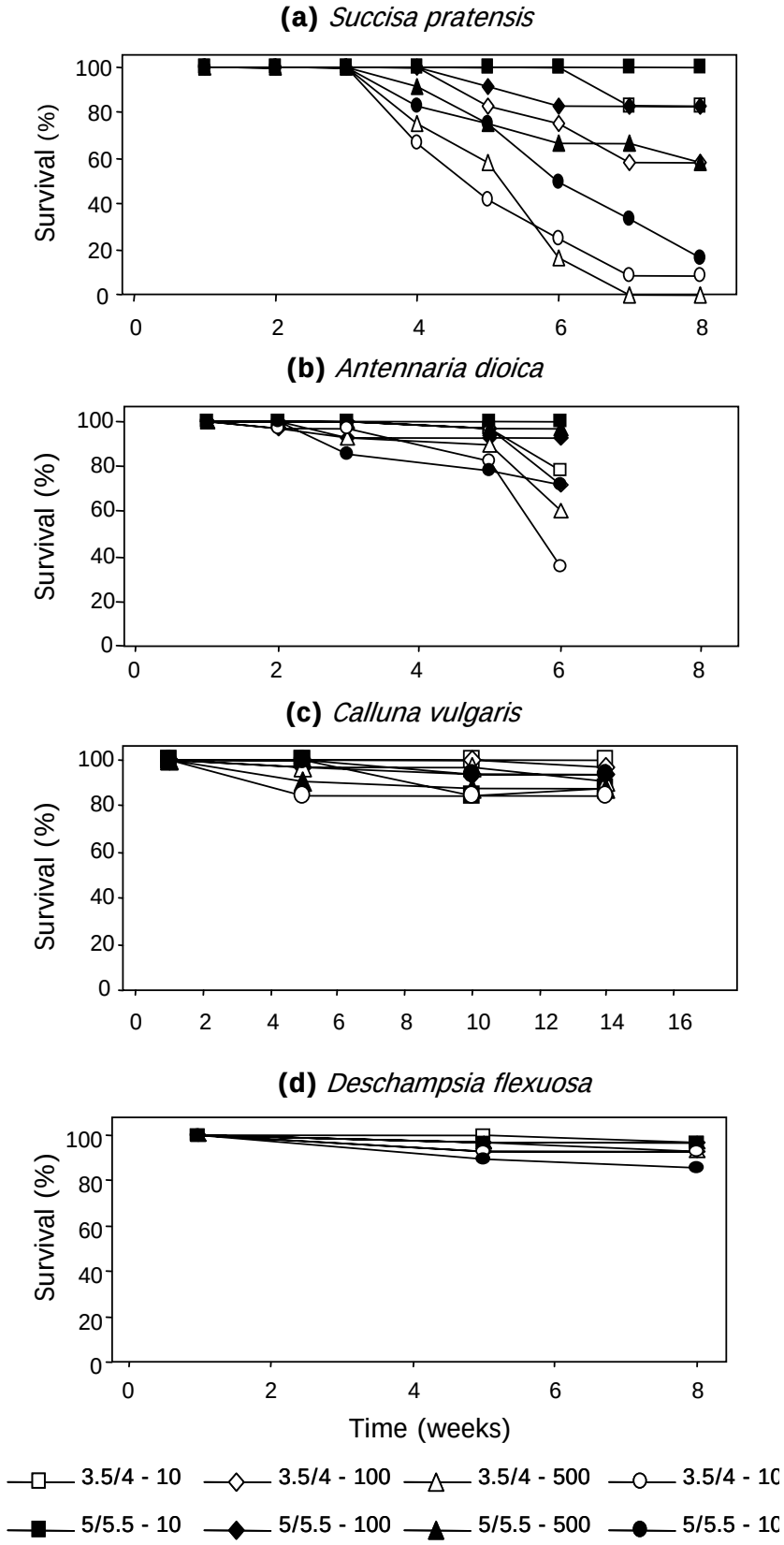

Figure 1: Survival (%) of *Succisa pratensis* **(a)**, *Antennaria dioica* **(b)**, *Calluna vulgaris* **(c),** and *Deschampsia flexuosa* **(d)** in time. *S. pratensis* was grown at pH levels 4 and 5.5, whereas *A. dioica*, *Calluna vulgaris,* and *D. flexuosa* were grown at pH 3.5 and 5. NH_4^+ concentrations were applied as 10, 100, 500, and 1000 µmol l^{-1}. Survival of *Gentiana pneumonanthe* was 100% in all treatments (not shown).

Table 3: Statistical results[1] of the effects of different NH_4^+ and pH levels on biomass of *Gentiana pneumonanthe*, *Succisa pratensis*, *Antennaria dioica*, *Calluna vulgaris* and *Deschampsia flexuosa*.

	Shoot				Root			
Source	*d.f.*	*Mean Square*	*F*	*p*†	*d.f.*	*Mean Square*	*F*	*p*†
Gentiana pneumonanthe								
NH_4	3	0.02	21.75	**0.000**	3	0.00	2.05	0.133
pH	1	0.04	46.27	**0.000**	1	0.01	20.15	**0.000**
NH_4 * pH	3	0.00	3.03	**0.049**	3	0.00	1.05	0.389
error	24	0.00			24	0.00		
Succisa pratensis								
NH_4	3	0.02	4.25	**0.025**	3	0.01	3.02	0.065
pH	1	0.01	0.98	0.338	1	0.00	0.26	0.618
NH_4 * pH	2	0.00	0.51	0.610	1	0.00	0.00	0.983
error	14	0.01			14	0.00		
Antennaria dioica								
NH_4	3	2.83	5.87	**0.004**	3	0.96	6.45	**0.003**
pH	1	50.47	104.53	**0.000**	1	20.62	138.46	**0.000**
NH_4 * pH	3	1.46	3.02	0.050	3	0.75	5.02	**0.008**
error	23	0.48			23	0.15		
Calluna vulgaris								
NH_4	3	9.86	11.16	**0.000**	3	4.45	1.08	0.376
pH	1	2.75	3.11	0.091	1	4.46	1.08	0.308
NH_4 * pH	3	2.50	2.83	0.060	3	8.01	1.95	0.149
error	24	0.88			24	4.12		
Deschampsia flexuosa								
NH_4	3	10.47	9.98	**0.000**	3	0.27	1.67	0.199
pH	1	0.29	0.27	0.606	1	0.02	0.14	0.709
NH_4 * pH	3	1.93	1.84	0.167	3	0.04	0.24	0.870
error	24	1.05			24	0.16		

[1] Only the between-subjects effects are shown.
† Significant *p*-values (at the $\alpha < 0.05$ level) are indicated in bold.

Nutrient composition and C/N content

Increased NH_4^+ concentrations resulted in a significant decrease of Ca and Mn concentrations in both shoots and roots of *G. pneumonanthe* and *D. flexuosa* and in shoots of *Calluna vulgaris* (**table 5**). K concentrations decreased with increasing NH_4^+ concentration as well, except for shoots of *G. pneumonanthe*, in which the concentrations increased. In *Calluna vulgaris*, K decreased in the roots with increasing NH_4^+ (from an average of 98 µmol g^{-1} DW, for both pH levels, to 75 µmol g^{-1} DW), but showed an optimum curve with increasing NH_4^+ in the shoots. The K

concentration was significantly higher in the 100 and 500 µmol l^{-1} NH_4^+ treatments (average of 237 µmol g^{-1} DW for both pH levels) compared to the treatments with 10 or 1000 µmol l^{-1} NH_4^+ (average of 183 µmol g^{-1} DW for both pH levels). Mg concentrations decreased with increasing NH_4^+ concentration only in roots of *G. pneumonanthe*, *Calluna vulgaris* and *D. flexuosa.* There were no differences measured in the shoots of these three species.
Other negative effects of NH_4^+ concentration could be found for P in shoots of *Calluna vulgaris* (lowest value was 87 µmol g^{-1} DW in the 1000 µmol l^{-1} NH_4^+/pH 3.5 treatment) and roots of *G. pneumonanthe* (50 µmol g^{-1} DW in the 1000 µmol l^{-1} NH_4^+/pH 3.5 treatment). A significant increase in S concentration with increasing NH_4^+ concentrations was found in the shoots of *G. pneumonanthe*, *Calluna vulgaris* and *D. flexuosa* and in the roots of *D. flexuosa*. N content of all tissues increased significantly with increasing NH_4^+ concentrations in all species, except for shoots of *G. pneumonanthe*. In *D. flexuosa* tissue and in the roots of *G. pneumonanthe* an increase in C content was measured with increasing NH_4^+ concentrations.
The pH level of the treatments did not affect *D. flexuosa* except for the N content of the roots. In contrast, higher values of almost all nutrients were found at the highest pH level (**table 5**) in shoots of *Calluna vulgaris* and both shoots and roots of *G. pneumonanthe*. Mn concentrations were also higher at the higher pH level in the roots of *Calluna vulgaris.* C content in both shoots and roots of *G. pneumonanthe* was significantly lower at pH 4. A pH * NH_4^+ interaction effect was measured for the K concentration in the roots of *Calluna vulgaris* and for the N content of the shoots.

DISCUSSION

Effects of NH_4^+ and pH on the survival and growth of heathland species

A. dioica and *S. pratensis* were expected to be acid-sensitive species. Acid-sensitive species are naturally found on slightly buffered soils (pH 4.5-6) where mineral N is available in the form of nitrate (NO_3^-) as well as in the form of NH_4^+. These species will be greatly affected by increased NH_4^+ concentrations as they prefer NO_3^- over NH_4^+ (Falkengren-Grerup and Lakkenborg-Kristensen, 1994; Falkengren-Grerup, 1995). Our results support the hypothesis that *A. dioica* and *S. pratensis* are acid-sensitive herbaceous species, suffering from combined effects of high NH_4^+ concentrations and low pH. NH_4^+ toxicity was observed for *A. dioica* and *S. pratensis* at pH levels that resembled weakly buffered soil conditions (pH 5 and 5.5 treatments). These negative effects of NH_4^+ nutrition on survival and growth of *S. pratensis* and on survival of *A. dioica* were even more pronounced at pH levels resembling acidified soil conditions (pH 3.5 and pH 4 treatments), as was illustrated by a slight NH_4^+ * pH interaction effect (**table 2**).
Biomass of *A. dioica* was largely determined by the pH of the medium (**figure 2**) with a lower total biomass in the acidic treatment. No effect of NH_4^+ on root biomass was observed at pH 3.5, as opposed to a significant negative NH_4^+ effect at pH 5, which is illustrated by the NH_4^+ * pH interaction effect (**table 3**). This suggests that growth of *A. dioica* is affected by NH_4^+ concentrations at the higher pH levels, whereas at pH 3.5 growth conditions are detrimental, irrespective of NH_4^+ levels. In contrast, the

species which were classified as acid-tolerant species, *D. flexuosa* and *Calluna vulgaris,* demonstrated an increased total biomass with increasing NH_4^+ concentrations. Only at low pH, *Calluna vulgaris* suffered from very high NH_4^+ concentrations (viz. 1000 µmol l^{-1}), indicated by a lower biomass and a slight NH_4^+ * pH interaction effect on survival. This shows that strongly increased NH_4^+ concentrations (i.e. eutrophication) in combination with low pH (i.e. acidification) might well affect the performance of *Calluna vulgaris*. An increased biomass of both acid-tolerant species with increasing NH_4^+ concentrations suggested N limitation at the lower NH_4^+ concentrations.

Table 4: Statistical results[1] of the effects of different ammonium (NH_4^+) and pH levels on internal pH of *Gentiana pneumonanthe*, *Succisa pratensis*, *Antennaria dioica*, *Calluna vulgaris* and *Deschampsia flexuosa*.

	Shoot				Root			
Source	*d.f.*	*Mean Square*	*F*	*p*†	*d.f.*	*Mean Square*	*F*	*p*†
Gentiana pneumonanthe								
NH_4	3	0.38	4.90	**0.009**	3	0.50	37.88	**0.000**
pH	1	0.10	1.30	0.266	1	0.00	0.02	0.879
NH_4 * pH	3	0.00	0.05	0.986	3	0.02	1.60	0.216
error	24	0.08			24	0.01		
Succisa pratensis								
NH_4	3	0.66	21.42	**0.000**	3	0.78	25.09	**0.000**
pH	1	0.00	0.16	0.693	1	0.15	4.66	0.050
NH_4 * pH	2	0.05	1.47	0.263	2	0.09	2.99	0.086
error	14	0.03			13	0.03		
Antennaria dioica								
NH_4	3	0.93	13.53	**0.000**	3	0.49	3.84	**0.021**
pH	1	13.23	193.25	**0.000**	1	9.47	73.84	**0.000**
NH_4 * pH	3	0.11	1.58	0.219	3	1.37	10.65	**0.000**
error	26	0.07			26	0.13		
Calluna vulgaris								
NH_4	3	0.06	1.71	0.189	3	0.14	0.85	0.479
pH	1	0.00	0.01	0.919	1	0.01	0.03	0.860
NH_4 * pH	3	0.02	0.53	0.664	3	0.38	2.33	0.097
error	27	0.04			27	0.16		
Deschampsia flexuosa								
NH_4	3	0.34	2.83	0.057	3	2.39	17.47	**0.000**
pH	1	0.28	2.32	0.139	1	0.08	0.58	0.451
NH_4 * pH	3	0.23	1.94	0.148	3	0.41	3.01	**0.048**
error	27	0.12			27	0.14		

[1] Only the between-subjects effects are shown.
† Significant *p*-values (at the $\alpha < 0.05$ level) are indicated in bold.

The herbaceous species *G. pneumonanthe* was expected to be slightly acid-tolerant. This was illustrated by an increase in biomass and survival of *G. pneumonanthe* with increasing NH_4^+ concentrations. The species was able to withstand low pH in combination with high NH_4^+ concentrations. At high NH_4^+ concentrations (500 and 1000 µmol l^{-1}) biomass did not increase or decrease, indicating that other nutrients may have become limiting.
Calluna vulgaris, *D. flexuosa* and *G. pneumonanthe* are naturally found on slightly acidic to more acidic soils and might be adapted to NH_4^+ nutrition, since NH_4^+ is the common form of inorganic N at low soil pH (Gigon and Rorison, 1972; Troelstra *et al.*, 1990). This might explain why these species, apart from only *Calluna vulgaris* at low pH, are not affected by high NH_4^+ concentrations. Data from several studies have shown that acid-tolerant species can tolerate high NH_4^+ concentrations (Gigon and Rorison, 1972; Falkengren-Grerup and Lakkenborg-Kristensen, 1994; Falkengren-Grerup, 1995).
Although survival of *G. pneumonanthe* did not differ from that of *Calluna vulgaris* and *D. flexuosa* in different NH_4^+ and pH treatments, negative effects of acidification on biomass were observed. Based on field data, *G. pneumonanthe* can be regarded to be more sensitive to acidic conditions than *Calluna vulgaris* and *D. flexuosa*. Since there were no differences for survival in this study, this sensitivity may well be caused by indirect pH effects via increased Al toxicity. From earlier studies it is known that increased acidification results in increased Al concentrations, which can be toxic for many herbaceous species (Kinraide, 1997; **chapter 6** of this thesis). In this experiment, plants were grown on hydroculture, in which Al concentrations were kept constantly low. The results of this study therefore do not allow drawing any conclusions concerning indirect pH effects such as Al toxicity.

Internal pH differences and nutrient content

Many studies have reported on the mechanisms for NH_4^+ toxicity (for a review, see Britto and Kronzucker, 2002). One of these mechanisms is the decrease of the internal pH of the plant at elevated NH_4^+ concentrations in the rhizosphere (Raven, 1986; Marschner, 1995). In the present study a decrease in internal pH at increasing NH_4^+ concentrations was observed for most species in both shoots and roots, which is in accordance with other studies that found proton production when NH_4^+ is assimilated (Raven and Smith, 1976; Raven, 1986; Gerendás, 1990; Britto and Kronzucker, 2002). The proton production results in internal acidification and protons have to be excreted or neutralised to maintain cell pH homeostasis. This leads to the acidification of the rhizosphere (e.g. Findenegg, 1987; Van Beusichem *et al.*, 1988; Goodchild and Givan, 1990; De Graaf *et al.*, 2000).
In this study we found a significant pH effect and NH_4^+ * pH interaction effect on the internal pH of roots of the acid-sensitive *A. dioica* (**table 4**), but not of *S. pratensis*. For the latter species, the effect of a low pH was too dominant (**figure 2**). The effects of decreasing pH and increasing NH_4^+ in the medium on the internal pH of *A. dioica* were visible in both shoots and roots. The NH_4^+ * pH interaction effect on internal pH of the roots and shoots illustrates the sensitivity to low pH levels and increasing

NH_4^+ concentrations. These effects resemble those found for the biomass of *A. dioica* (**figure 2; table 3**).
Surprisingly, internal pH values decreased with increasing NH_4^+ concentrations for the acid-tolerant *G. pneumonanthe* and *D. flexuosa* as well, despite the fact that survival and biomass increased (**tables 2 and 3**). These species seemed to be tolerant for low internal pH levels, suggesting that internal pH may not be a suitable indicator for NH_4^+ toxicity and/or plant performance. This is consistent with an earlier study by Paulissen *et al.* (2004) who did not find any serious physiological problems for *Polytrichum commune* when internal pH decreased due to NH_4^+ nutrition.
Increased NH_4^+ concentrations resulted in an increase in N concentration in all tissues of all species, except for the shoots of *G. pneumonanthe*. This indicates an increased N uptake with increasing NH_4^+ in the treatments for these species. In addition, N:P ratios in all species were between 0 and 3 g g^{-1} and remained well below the suggested minimal value for P limitation (Koerselman and Meuleman, 1996). NH_4^+ uptake resulted in a decrease in cation uptake (**table 5**), which is known to be a possible NH_4^+ toxicity mechanism, as it can result in cation deficiency (Kirkby, 1968; Van Beusichem *et al.*, 1988). In addition, a study by De Graaf *et al.* (1998) showed Ca and Mg deficiency due to reduced uptake in *C. dissectum* grown at high NH_4^+ concentrations and low pH. Lucassen *et al.* (2003) found similar results in media with both high and low pH, and concluded that NH_4^+ was responsible for the reduced cation content. Cation concentration (Ca, Mg, K, Mn) of both the acid-tolerant species *Calluna vulgaris* and *D. flexuosa* and the supposed slightly acid-tolerant *G. pneumonanthe* was generally reduced at high levels of NH_4^+. However, these effects did not lead to a reduced performance. On the contrary, biomass of these three species increased with increasing NH_4^+. Only in the shoots of *Calluna vulgaris* treated with high NH_4^+ concentrations indications of chlorosis were found indicated by a light green colour (data not shown). For *G. pneumonanthe* and *D. flexuosa* no indications of cation deficiency could be noted. The concentration of S in the shoots of *G. pneumonanthe*, *Calluna vulgaris* and *D. flexuosa* increased with increasing NH_4^+ concentrations. This is in agreement with studies by Kirkby (1968) and van Beusichem *et al.* (1988), who found an increased uptake of anions including sulfate (SO_4^{2-}) with increasing NH_4^+ nutrition. This enhanced uptake was attributed to the replacement of NO_3^- reduction with SO_4^{2-} reduction, which is necessary for the regulation of the pH-stat mechanisms, when NO_3^- becomes limiting.
Next to the effects of NH_4^+, a decrease in external pH resulted in a decreased uptake of cations and reduced C concentration in the roots and shoots of *G. pneumonanthe*. This effect was also visible for the cation concentrations in the shoots of *Calluna vulgaris*. These results are consistent with earlier studies, in which a decrease in the pH of the rhizosphere and apoplast was found to decrease cation uptake, resulting in cation deficiencies of the plant (Van Beusichem *et al.*, 1988; Boxman *et al.*, 1991; Marschner, 1995). Clearly, the acid-tolerant species *D. flexuosa* was not affected by the external pH level apart from the N concentration in the roots. Shoots of *Calluna vulgaris*, however, also supposed to be acid-tolerant, contained more cations at the high pH level compared to the tissue at low pH level.

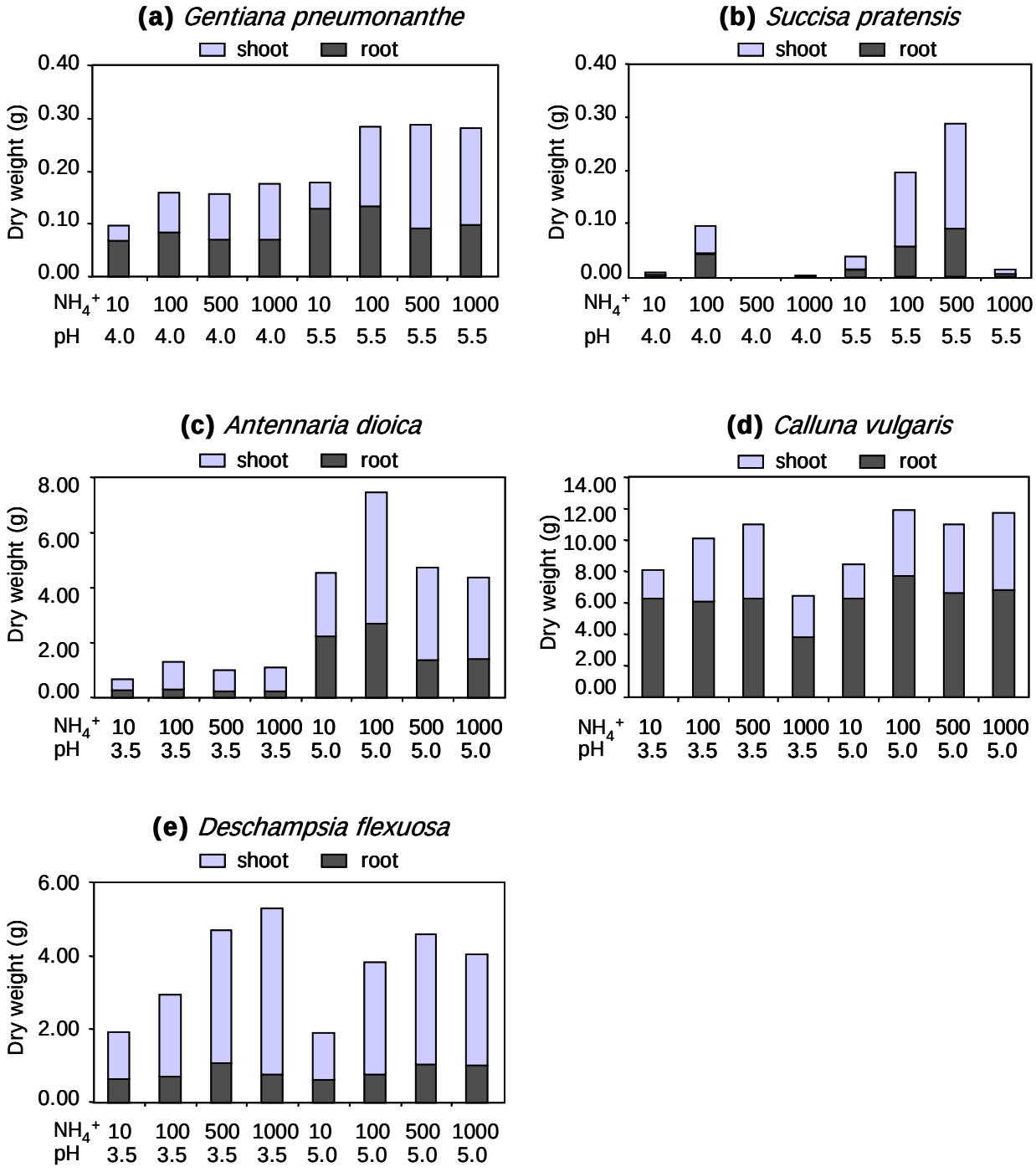

Figure 2: Biomass of shoots and roots of *Gentiana pneumonanthe* **(a)**, *Succisa pratensis* **(b)**, *Antennaria dioica* **(c)**, *Calluna vulgaris* **(d)**, and *Deschampsia flexuosa* **(e)** at the end of the experimental period. Plants were pooled per container in order to determine biomass. NH_4^+ concentrations were applied as 10, 100, 500, and 1000 µmol l^{-1}. *G. pneumonanthe* and *S. pratensis* were grown at pH levels 4 and 5.5, whereas *A. dioica*, *Calluna vulgaris*, and *D. flexuosa* were grown at pH 3.5 and 5.

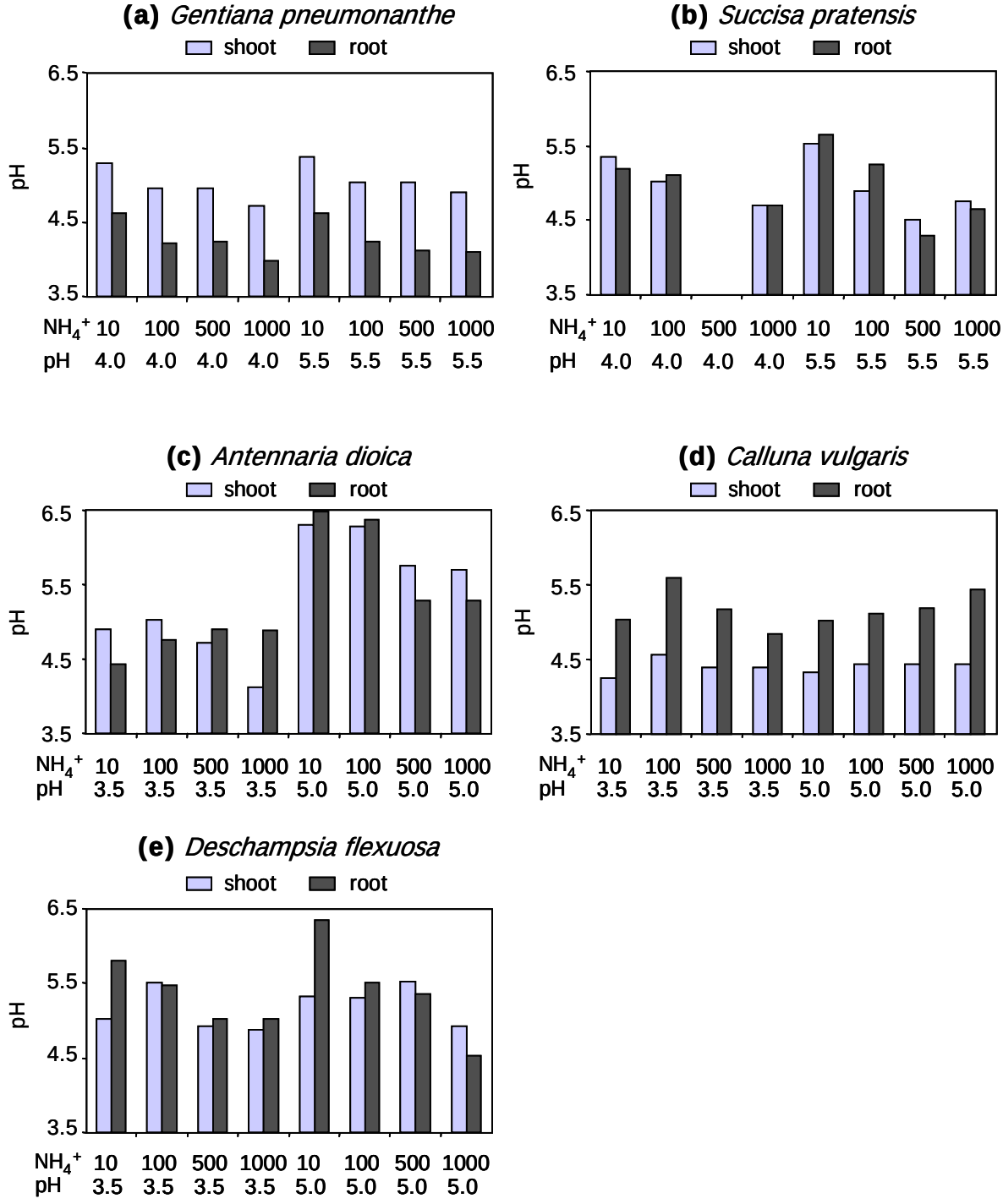

Figure 3: Internal pH values of shoots and roots of *Gentiana pneumonanthe* **(a)**, *Succisa pratensis* **(b)**, *Antennaria dioica* **(c)**, *Calluna vulgaris* **(d)** and *Deschampsia flexuosa* **(e)**. NH_4^+ concentrations were applied as 10, 100, 500, and 1000 µmol l^{-1}. *G. pneumonanthe* and *S. pratensis* were grown at pH levels 4 and 5.5, whereas *A. dioica*, *Calluna vulgaris*, and *D. flexuosa* were grown at pH 3.5 and 5.

As was mentioned before, *Calluna vulgaris* did not show negative effects in survival or biomass related to external pH, which suggests that the effect of external pH on nutrient content did not lead to cation deficiencies. However, the combination of a low pH and very high NH_4^+ concentration might lead to serious deficiencies in time. Shoots of *Calluna vulgaris* grown at 1000 µmol l^{-1} NH_4^+ showed indications of chlorosis (data not shown). As *Calluna vulgaris* responded slowly to subjected treatments, the experimental period of this study might be too short to record these effects; such effects may, however, have a significant impact in latter stages.
Lack of material due to high mortality and poor growth prevented us from analysing *S. pratensis* plants for nutrient content. It is, however, likely that cation deficiency was responsible for the decline of this species on high NH_4^+ and low pH medium. Such effects were earlier shown for *C. dissectum* (De Graaf *et al.*, 1998).

Implications for biodiversity in heathlands

Plant diversity is rapidly declining in heathlands and matgrass swards in Western Europe and this may be the result of increased N-deposition, which is causing acidification of the soil (Roelofs, 1986; Aerts *et al.*, 1990; Bobbink *et al.*, 1998; Lee and Caporn, 1998; Krupa, 2003). In earlier studies it was found that high NH_4^+ concentrations are detrimental for germination, growth and survival of several (rare) heathland species (De Graaf *et al.*, 1998; Dorland *et al.*, 2003) and these effects may be exacerbated at low soil pH (Lucassen *et al.*, 2003). In degraded acidified heathlands, soil solution pH ranged between 3.8 and 4.5, and NH_4^+ concentrations ranged between 300 and 650 µmol kg^{-1} DW (De Graaf *et al.*, 1994; Roelofs *et al.*, 1996; Dorland *et al.*, 2003). It was expected that the presence and decline of species in heathlands was correlated with the ranges of both NH_4^+ and pH. In this study we were interested in the specific interaction effects between NH_4^+ and pH on the performances of different plant species. In order to test this, we used a hydroponic approach with NH_4^+ and pH values comparable to those found in the field. Hydroponic studies provide a useful tool for investigating plant responses to specific dose dependent factors such as the NH_4^+ concentration and the pH of the medium. However, these types of experiments do not fully reflect the situations that occur in the field. For instance, by using hydroponic studies, the potential role of mycorrhiza, assimilation of nutrients via shoots and uptake kinetics are not included. Also, the concentrations of all elements in the growth medium are kept constant, which does not resemble realistic field situations. However, hydroponic techniques allowed us to determine the plant responses at different controlled NH_4^+ concentrations and pH levels in a direct and straightforward manner, including measuring the interaction of both independent variables.
The results of this study show that the NH_4^+ concentration and pH of the solution can have strong determining and interacting effects on the vegetation. These variables might also affect the composition of heathlands, with the reason for the decline varying per species.

Table 5: Statistical results of a General Linear Model (GLM) procedure on nutrient content of *Calluna vulgaris*, *Deschampsia flexuosa and Gentiana pneumonanthe* with ammonium and pH levels as fixed factors. Results for *Antennaria dioica* and *Succisa pratensis* cannot not be shown, as the results did not allow for statistical analysis due to high mortality and lack of material for the analyses.

	Root†#								Shoot†#							
	Ca	Mg	K	Mn	S	P	C	N	Ca	Mg	K	Mn	S	P	C	N
Calluna vulgaris																
pH	*ns*	*ns*	*ns*	** (+)	*ns*	*ns*	*ns*	*ns*	* (+)	* (+)	* (+)	* (+)	* (+)	*ns*	*ns*	* (opt.)
NH_4	*ns*	* (-)	** (-)	*** (-)	*ns*	*ns*	*ns*	*** (+)	** (-)	ns	*** (opt.)	** (-)	*** (+)	* (-)	ns	*** (+)
pH * NH_4	*ns*	*ns*	*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*
Deschampsia flexuosa																
pH	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	* (-)	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
NH_4	* (-)	* (-)	* (-)	*** (-)	*** (+)	*ns*	* (+)	** (+)	*** (-)	ns	** (-)	*** (-)	*** (+)	*ns*	*** (+)	*** (+)
pH * NH_4	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Gentiana pneumonanthe																
pH	*** (+)	*** (+)	*** (+)	*** (+)	*** (+)	*** (+)	** (-)	*ns*	*** (+)	*** (+)	*** (+)	*** (+)	*** (+)	*** (+)	** (-)	*ns*
NH_4	*** (-)	** (-)	*** (-)	*** (-)	*ns*	** (-)	* (+)	* (+)	** (-)	*ns*	** (+)	** (-)	*** (+)	*ns*	*ns*	*ns*
pH * NH_4	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

† Significance levels are indicated as follows: *ns* = not significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.
# Effects are indicated between brackets: **(+)** indicates a positive effect, **(-)** indicates a negative effect, **(opt.)** indicates an optimum with the lowest concentrations in the 10 or 1000 µmol l^{-1} NH_4^+ treatments and higher concentrations in the 100 and 500 µmol l^{-1} NH_4^+ treatments.

The decline of *A. dioica* from heathlands may be explained by negative effects of acidification and/or NH_4^+ toxicity at low pH and by NH_4^+ toxicity at high pH levels, wheras the decline of *G. pneumonanthe* in the Netherlands may be caused by factors other than NH_4^+ toxicity, as no detrimental effects of increased NH_4^+ concentrations on the performance of *G. pneumonanthe* were observed. Although this species is regarded to be acid-tolerant, indications for sensitivity to acidification were found. Other factors associated with low soil pH such as high Al concentrations might also be important. The results described in this study are in accordance with other studies which attributed the strong decline of many plant species of dry and wet heathlands and matgrass swards to both eutrophication and acidification (Roelofs, 1986; Aerts *et al.*, 1990; Lee and Caporn, 1998; Krupa, 2003). From our results, we may suggest that NH_4^+ toxicity can seriously affect plant diversity in acidified heathlands.
In contrast grasses such as *D. flexuosa*, can benefit from these soil conditions. The results show that this species can increase biomass at high concentrations of NH_4^+ nutrition, enabling this species to out-compete slower growing species. Therefore, competition for light and nutrients with grasses such as *D. flexuosa* may explain the strong decline in abundance and number of many herbaceous species.
Current restoration measures involve liming of the soil in order to reduce the effects of acidification. Liming results in increased soil pH and buffering cations (Dorland *et al.*, 2004), thereby reducing direct negative acidity effects on plants. In addition, Feng *et al.* (1992) found that proton release from the roots could be stimulated in an acid environment by the addition of Ca. Therefore, liming also reduces NH_4^+ toxicity symptoms resulting from decreased internal pH. In order to conserve acid-sensitive, herbaceous species in heathlands, restoration measures such as liming and removal of grasses by grazing and/or sod cutting, are a prerequisite.

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References

Aerts, R., Berendse, F. 1988. The effect of increased nutrient availability on vegetation dynamics in wet heathlands. *Vegetatio*, 76: 63-69.

Aerts, R., Berendse, F., De Caluwe, H., Schmitz, M. 1990. Competition in heathland along an experimental gradient of nutrient availability. *Oikos*, 57: 310-318.

Berendse, F., Aerts, R. 1984. Competition between *Erica tetralix* L. and *Molinia caerulea* (L.) Moench as affected by the availability of nutrients. *Acta Oecologia/Oecologia Plantarum*, 5: 3-14.

Bobbink, R., Heil, G.W., Raessen, M.B.A.G. 1992. Atmospheric deposition and canopy exchange processes in heathland ecosystems. *Environmental Pollution*, 75: 29-37.

Bobbink, R., Hornung, M., Roelofs, J.G.M. 1998. The effects of air borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, 86: 717-738.

Bobbink, R., Ashmore, M., Braun, S., Fluckiger, W., Van den Wyngaert, I.J.J. 2003. Empirical nitrogen critical loads for natural and semi-natural ecosystems: 2002 update. In: Achermann, B., Bobbink, R. (Eds.). *Empirical critical loads for nitrogen. Environmental Documentation No. 164. Air*. SAEFL, Berne, Switzerland, pp. 43-170.

Boxman, A.W., Krabbendam, H., Bellemakers, M.J.S., Roelofs, J.G.M. 1991. Effects of ammonium and aluminium on the development and nutrition of *Pinus nigra* in hydroculture. *Environmental Pollution*, 73: 119-136.

Boxman, A.W., Van der Ven, P.J.M., Roelofs, J.G.M. 1998. Ecosystem recovery after a decrease in nitrogen input to a Scots pine stand at Ysselsteyn, The Netherlands. *Forest Ecology Management*, 101: 155-163.

Britto, D.T., Kronzucker, H.J. 2002. NH_4^+ toxicity in higher plants: a critical review. *Journal of Plant Physiology*, 159: 567-584.

Carnol, M., Ineson, P., Dickinson, A.L. 1997. Soil solution nitrogen and cations influenced by $(NH_4)_2SO_4$ deposition in a coniferous forest. *Environmental Pollution*, 97: 1-10.

De Graaf, M.C.C., Bobbink, R., Roelofs, J.G.M., Verbeek, P.J.M. 1998. Differential effects of ammonium and nitrate on 3 heathland species. *Plant Ecology*, 135: 185-196.

De Graaf, M.C.C., Bobbink, R., Verhagen, R.C.M., Crins, E., Roelofs, J.G.M. 2000. Effects of nitrogen nutrition and aluminium on seedling development of *Arnica montana* L. and *Cirsium dissectum* (L.) Hill. In: *Exploring the Calcicole-Calcifuge gradient in Heathlands.* PhD Thesis. University of Nijmegen, Nijmegen, The Netherlands.

De Graaf, M.C.C., Verbeek, P.J.M., Cals, M.J.R., Roelofs, J.G.M. 1994. *Effectgerichte maatregelen tegen verzuring en eutrofiëring van matig mineraalrijke heide en schraallanden*. University of Nijmegen, Nijmegen, The Netherlands.

De Smidt, J.T. 1975. *Nederlandse heidevegetaties (Heathland vegetations of the Netherlands)*. PhD Thesis, Utrecht University, Utrecht, The Netherlands.

De Smidt, J.T. 1979. Origin and destruction of Northwest European heath vegetation. In: Wilmanns, O., Tüxen, R. (Eds.). *Werden und Vergehen von Pflanzengesellschaften*. J. Cramer, Vaduz, Liechtenstein, pp. 411-435.

Dijk, E., Eck, N. 1995. Ammonium toxicity and nitrate response of axenically grown *Dactylorhiza incarnata* seedlings. *New Phytologist*, 131: 361-367.

Dijk, E., Grootjans, A.P. 1998. Performance of four *Dactylorhiza* species over a complex trophic gradient. Acta Botanica Neerlandica, 47: 351-368.

Dorland, E., Bobbink, R., Messelink, J.H., Verhoeven, J.T.A. 2003. Soil ammonium accumulation after sod cutting hampers the restoration of degraded wet heathlands. *Journal of Applied Ecology*, 40: 804-814.

Dorland, E., Van den Berg, L.J.L., Van den Berg, A.J., Vermeer, M., Roelofs, J.G.M., Bobbink, R. 2004. The effects of sod cutting and additional liming on potential net nitrification in heathland soils. *Plant and Soil,* 265: 267-277.

Dueck T.A., Elderson J. 1992. Influence of NH_3 and SO_2 on the growth and competitive ability of *Arnica montana* L. and *Viola canina* L. *New Phytologist*, 122: 507-512.

Erisman J.W. 1990. Acid deposition in the Netherlands. RIVM, report 723001002, Bilthoven, The Netherlands.

Falkengren-Grerup, U. 1995. Interspecies differences in the preference of ammonium and nitrate in vascular plants. *Oecologia*, 102: 305-311.

Falkengren-Grerup, U., Lakkenborg-Kristensen, H. 1994. Importance of ammonium and nitrate to the performance of herb-layer species from deciduous forests in southern Sweden. *Environmental and Experimental Botany*, 34: 31-38.

Feng, Y., Schubert, S., Mengel, K. 1992. Effect of low root medium pH on net proton release, root respiration and root growth of Corn (*Zea mays* L.) and Broad bean (*Vicia faba* L.). *Plant Physiology*, 99: 415-421.

Findenegg, G.R. 1987. A comparative study on ammonium toxicity at different constant pH of the nutrient solution. *Plant and Soil*, 103: 239-243.

Gerendás, J., Ratcliffe, R.G., Sattelmacher, B. 1990. ^{31}P nuclear magnetic resonance evidence for differences in intracellular pH in the roots of maize seedlings grown with nitrate or ammonium. *Journal of Plant Physiology*, 137: 125-128.

Gigon, A., Rorison, I.H. 1972. The response of some ecologically distinct plant species to nitrate- and to ammonium-nitrogen. *Journal of Ecology*, 60: 93-102.

Gimingham, C.H. 1992. *The Lowland Heathland Management Handbook*. English Nature, Peterborough, United Kingdom.

Goodchild, J.A., Givan, C.V. 1990. Influence of ammonium and extracellular pH on the amino and organic-acid contents of suspension-culture cells of *Acer pseudoplatanus*. *Physiologia Plantarum*, 78: 29-37.

Grootjans, A.P., Sival, F.P., Stuyfzand, P.J. 1996. Hydro-geochemical analysis of a degraded dune slack. *Vegetatio*, 126: 27-38.

Heil, G.W., Diemont, W.H. 1983. Raised nutrient levels change heathland into grassland. *Vegetatio*, 53: 113-120.

Heijne, B., van Dam, D., Heil, G.W., Bobbink, R. 1996. Acidification effects on vescular-arbuscular mycorrhizal (VAM) infection, growth and nutrient uptake of established heathland herb species. *Plant and Soil*, 179: 197-206.

Houdijk, A.L.F.M., Verbeek, P.J.M., Van Dijk, H.F.G., Roelofs, J.G.M. 1993. Distribution and decline of endangered herbaceous heathland species in relation to the chemical composition of the soil. *Plant and Soil*, 148: 137-143.

Kinraide, T.B. 1997. Reconsidering the rhizotoxicity of hydroxyl, sulphate and fluoride complexes of aluminium. *Journal of Experimental Botany,* 48: 1115-1124.

Kirkby, E.A. 1968. Influence of ammonium and nitrate nutrition on the cation-anion balance and nitrogen and carbohydrate metabolism of white mustard plants grown in diluted solutions. *Soil Science,* 105: 133-141.

Koerselman, W., Meuleman, A.F.M. 1996. The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology,* 33: 1441-1450.

Kreutzer, K., Beier, C., Bredemeier, M., Blanck, K., Cummins, T., Farrell, E.P., Lammersdorf, N., Rasmussen, L., Rothe, A., de Visser, P.H.B., Weiss, W., Weiss, T., Xu, Y.J. 1998. Atmospheric deposition and soil acidification in five coniferous forest ecosystems: a comparison of the control plots of the EXMAN sites. *Forest Ecology and Management,* 107: 327.

Krupa, S.V. 2003. Effects of atmospheric ammonia (NH_3) on terrestrial vegetation; a review. *Environmental pollution,* 124: 179-221.

Lamers, L.P.M., Van Roozendaal, S.M.E., Roelofs, J.G.M. 1998. Acidification of freshwater wetlands: combined effects of non-airborne sulphur pollution and desiccation. *Water, Air and Soil Pollution,* 105: 95-106.

Lee, J.A. Caporn, S.J.M. 1998. Ecological effects of atmospheric reactive nitrogen deposition on semi-natural terrestrial ecosystems. *New Phytologist,* 139: 127-134.

Lucassen, E.C.H.E.T., Bobbink, R., Smolders, A.J.P., Van der Ven, P.J.M., Lamers, L.P.M., Roelofs, J.G.M. 2003. Interactive effects of low pH and high ammonium levels responsible for the decline of *Cirsium dissectum* (L.) Hill. *Plant Ecology,* 165: 45-52.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants* (2nd edition). Academic Press, London, United Kingdom.

Mehrer, I., Mohr, H. 1989. Ammonium toxicity: description of the syndrome in *Sinapsis alba*

and the search for its causation. *Physiologia Plantarum,* 77: 545-554.
Paulissen, M.P.C.P., van der Ven, P., Dees, A., Bobbink, R. 2004. Differential effects of nitrate and ammonium on three fen bryophyte species in relation to pollutant nitrogen input. *New Phytologist* 164: 451-458.
Pitcairn, C.E.R., Fowler, D., Grace, J. 1991. *Changes in Species Composition of Semi-Natural Vegetation Associated With the Increase in Atmospheric Inputs of Nitrogen*. Institute of Terrestrial Ecology/NERC, Penicuik, United Kingdom.
Raven, J.A. 1986. Biochemical disposal of excess H^+ in growing plants? *New Phytologist,* 104:175-206.
Raven, J.A., Smith, F.A. 1976. Nitrogen assimilation and transport in vascular plants in relation to intracellular pH regulation. *New Phytologist,* 54: 249-274.
Roelofs, J.G.M. 1986. The effect of airborne sulphur and nitrogen deposition on aquatic and terrestrial heathland vegetation. *Experientia,* 42: 372-377.
Roelofs, J.G.M. 1993. De fragiele balans tussen verzuring en verbasing in blauwgraslanden. In: Weeda E.J. (Ed.). *Blauwgraslanden in Twente: schatkamers van het natuurbehoud*. KNNV Uitgeverij, Utrecht, The Netherlands, pp. 32-38.
Roelofs, J.G.M., Kempers, A.J., Houdijk, L.F.M., Jansen, J. 1985. The effect of air-borne ammonium sulphate on *Pinus nigra* var. *maritima* in the Netherlands. *Plant and Soil,* 84: 45-56.
Roelofs, J.G.M., Bobbink, R., Brouwer, E., De Graaf, M.C.C. 1996. Restoration ecology of aquatic and terrestrial vegetation on non-calcareous sandy soils in the Netherlands. *Acta Botanica Neerlandica,* 45: 517-541.
Runhaar, J., Van Gool, C.R., Groen, C.L.G. 1996. Impact of hydrological changes on nature conservation areas in the Netherlands. *Biological Conservation,* 76: 269-276.
Troelstra, S.R., Wagenaar, R., De Boer, W. 1990. Nitrification in Dutch heathland soils. 1. General soil characteristics and nitrification in undisturbed soil cores. *Plant and Soil,* 127: 179-192.
Van Beusichem, M.L., Kirkby, E.A., Baas, R. 1988. Influence of nitrate and ammonium nutrition on the uptake, assimilation and distribution of nutrients in *Ricinus communis*. *Plant Physiology,* 86: 914-921.
Van Breemen, N., Van Dijk, H.F.G. 1988. Ecosystem effects of atmospheric deposition of nitrogen in the Netherlands. *Environmental Pollution,* 54: 249-274.
Van Dam, D., Van Dobben, H.F., Ter Braak, C.F.J., De Wit, T. 1986. Air pollution as a possible cause for the decline of some phanerogamic species in the Netherlands. *Vegetatio*, 65: 47-52.
Van der Meijden, R. 2002. *Heukel's Flora Van Nederland* (22nd edition). Wolters-Noordhoff B.V., Groningen, The Netherlands.
Van Dobben, HF. 1991. Integrated effects (low vegetation). In: Heij, G.J., Schneider, T. (Eds.). *Final report of the Dutch priority programme on acidification*. Elsevier, Amsterdam, The Netherlands, pp. 464-524.
Vonk, M., van der Hoek, D.C.J., van de Meent, D., Wortelboer, F.G., Alkemade, J.R.M. 2001. *Calculation of the effects of the environmental quality on nature for application to the Fifth National Environmental Outlook*. RIVM report 408129017, Bilthoven, The Netherlands.
Webb, N.R. 1998. The traditional management of European heathlands. *Journal of Applied Ecology*, 35: 987-990.
Webb, N.R. 2002. Atlantic heathlands. In: Perrow M.R., Davy A.J. (Eds.). *Handbook of ecological restoration. Volume 2: restoration in practice*. Cambridge University Press, Cambridge, United Kingdom, pp. 401-418.

Chapter 4

REDUCED VERSUS OXIDISED NITROGEN: IMPLICATIONS FOR THE DEVELOPMENT OF DRY HEATHLAND VEGETATION, BASED ON A MESOCOSMS STUDY

Leon J.L. van den Berg, Cornelis J.H. Peters and Jan G.M. Roelofs

Summary Studies on the effects of different N forms in N deposition on changes in the vegetation composition are rare, but are necessary as large differences in both reduced and oxidised N in N deposition exist regionally and internationally. Different N forms may affect vegetation types differently and this may have serious consequences for nature management and policy regarding air pollution control. We investigated the effects of different ratios of reduced/oxidised nitrogen in soils with a surplus of nitrogen on plant growth responses and vegetation compositions of heathlands and species-rich grasslands. For this, we used large mesocosms in a greenhouse with four herbaceous species, *Antennaria dioica*, *Arnica montana*, *Gentiana pneumonanthe* and *Thymus serpyllum*, as well as the grasses *Danthonia decumbens*, *Deschampsia flexuosa and Nardus stricta and* the shrub *Calluna vulgaris*. The results show that 3 of the herbaceous species and the grass *D. decumbens* preferred low NH_4^+/NO_3^- ratios and hence were characterised by a negative correlation between NH_4^+/NO_3^- ratio in the soil and biomass and survival, whereas the grasses *N. stricta* and *D. flexuosa* showed no correlation with NH_4^+/NO_3^- ratio in the soil, or a slight (non-significant) positive correlation. We suggest that the decline of herbaceous species in heathlands due to atmospheric N deposition is largely due to increased NH_4^+ concentrations and hence increased NH_4^+/NO_3^- ratios in the soil.

Synthesis and applications The decline of species in poorly buffered systems was shown to be correlated to severe acidification and the ammonium concentration in the pore water. We found that atmospheric N deposition and particularly the form in which N is deposited, affects the ecosystem drastically and reduced N (elevated NH_4^+/NO_3^- ratios) was a strong determining factor in vegetation composition, especially in acidified soils. For policy and nature management decisions this is of great importance, as different forms of N dominate in different areas and thus the vegetation responses will vary as a result of this.

INTRODUCTION

Heathlands and species-rich grasslands are vegetation types characterised by poor soil fertility and low productivity and are dominated by small-leaved evergreen dwarf shrubs (Gimingham *et al.*, 1979). These vegetations can be found in various parts of the world, especially in mountainous habitats and are common in the Atlantic and sub-Atlantic regions of Western Europe. Lowland heathlands and species-rich grasslands were once a common semi-natural vegetation type in the temperate regions of Western Europe maintained by mowing, sheep grazing, burning and sod removal, which prevented succession towards woodlands (Heil and Aerts, 1993). From the beginning of the 20th century, however, heathlands and species-rich grasslands in Western Europe started to decline, mainly as a result of changes in land use, urbanisation and conversion to agricultural land (De Smith 1975, 1979; Webb, 2002). Despite many conservation programmes and the incorporation of heathlands in nature reserves the decline of heathlands has continued over the last 50 years. This has mainly been attributed to habitat fragmentation, changes in hydrology and an increased atmospheric deposition of nitrogen (N) and sulphur (S) compounds, resulting in eutrophication and acidification of the soil (Heil and Diemont, 1983; Galloway, 1995; Roelofs *et al.*, 1996; Bobbink *et al.*, 1998).

From the early 1980s it became clear that N deposition was a major determining factor in the species composition of these vegetation types. It was suggested that atmospheric N deposition results in grasses becoming dominant and species-rich dwarf shrub vegetations dominated by *Calluna vulgaris* developing into species-poor vegetations dominated by grasses such as *Molinia caerulea* (on formerly wet and dry heaths) and *Deschampsia flexuosa* (on formerly dry heaths; Heil and Diemont, 1983; Roelofs, 1986; Aerts and Heil, 1993; Swertz *et al.*, 1996). Results of many N enrichment studies on species-rich grasslands and heathlands support this view and generally show an increased growth of grasses with increased N load at the expense of herbaceous species (see, e.g., Morecroft *et al.*, 1994; Kellner and Redbo-Tortensson, 1995; Carroll *et al.*, 1997; Tomassen *et al.*, 2003).

Atmospheric N deposition consists largely of reduced N (NH_x), mainly originating from agricultural sources and oxidised N (NO_y), mainly originating from fuel combustion (Galloway, 1995). In large parts of Western Europe (e.g. Germany, The Netherlands, large parts of Great Britain) N is mostly deposited in the reduced form, whereas in other parts (e.g. parts of Scandinavia) it is oxidised N that dominates (Bobbink *et al.*, 1992; Eerens *et al.*, 2001; Galloway and Cowling, 2002). A recent comparative study by Smart *et al.* (2004) a clear correlation found between the form of N deposited on species-rich grasslands, heaths and bogs and changes in the vegetation. In the UK, deposition of reduced N, but not of oxidised N, was spatially correlated with vegetation changes over recent decades. Detailed field studies carried out in oak forests in southern Scandinavia have shown that changes in the species composition of ground flora are closely correlated to the dominant N form in the soils and to soil processes like nitrification and mineralization, both of which are related to soil acidity (Diekmann and Falkengren-Grerup, 1998, 2002; Falkengren-Grerup and Schöttelndreier, 2004).

High concentrations of NH_4^+ have been found to be toxic to many herbaceous plants, whereas increased concentrations of NO_3^- were found to stimulate growth (Gigon and Rorison, 1972; De Graaf *et al.*, 1998). Several studies indicated that herbaceous plants species from species-rich grasslands preferred NO_3^-, whereas many grasses and Ericoid species preferred NH_4^+ as their main N source (De Graaf *et al.*, 1998; Falkengren-Grerup and Schöttelndreier, 2004). The vulnerability of herbaceous plant species to elevated NH_4^+ concentrations has been attributed to a reduced base cation uptake, leading to cation deficiency (Bennet and Adams, 1969; Polle *et al.*, 1994; De Graaf *et al.*, 1998). Recent studies using hydroponics found that the herbaceous species *Gentiana pneumonanthe*, *Antennaria dioica*, *Succisa pratensis* and *Cirsium dissectum* were sensitive to high NH_4^+ concentrations in combination with low pH (3.5-4) levels (Lucassen *et al.*, 2003; **chapter 3** of this thesis). In contrast, acid-tolerant grasses such as *D. flexuosa* did not show such sensitivity, indicating that species from acidic and slightly buffered ecosystems in particular might be dramatically affected by a shift from an NO_3^- dominated system towards an NH_4^+ dominated ecosystem in acidified conditions (**chapter 3**).

Although the effects of atmospheric N deposition on vegetation development have been extensively investigated (see, e.g., Bobbink and Heil, 1993; Power *et al.*, 1998; Caroll *et al.*, 2003), there have been few reports on the effects of atmospheric deposition of reduced (NH_4^+) versus oxidised (NO_3^-) nitrogen, i.e. of different NH_4^+/NO_3^- ratios, on the growth and development of acid-sensitive vegetation types. As the NH_4^+/NO_3^- ratios of atmospheric N deposition change over time and can vary markedly per region, it is important to know how and to what extent this affects the vegetation development. Since high atmospheric N deposition in large parts of Western Europe is resulting in acidification and a shift towards higher NH_4^+/NO_3^- ratios, acidic and acid-sensitive vegetation types such as the species-rich grasslands and heathlands are expected to be vulnerable to the combined effects of increased NH_4^+/NO_3^- ratios and acidification of the soil.

To investigate the effect of different NH_4^+/NO_3^- ratios in the soil in response to different NH_4^+/NO_3^- ratios in atmospheric N deposition on species-rich grasslands and heathlands we used mesocosms filled with mineral soil from a heathland, to which artificial rain with a high total N deposition with different NH_4^+/NO_3^- deposition ratios was applied. Lime was added to one half of the mesocosms in order to investigate the interaction between pH of the soil pore water, NH_4^+/NO_3^- ratios and plant growth responses. Four herbaceous species and four grasses were planted in each mesocosm. The biomass, mortality and the chemical composition of the plants were measured to estimate fitness and survival. The effects of different NH_4^+/NO_3^- ratios in the soil on heathland vegetation development were analysed using regression analysis. We hypothesised that acidification and high NH_4^+/NO_3^- ratios would negatively affect the survival and fitness of the herbaceous species *A. dioica*, *A. montana*, *G. pneumonanthe*, *T. serpyllum* and the grass *D. decumbens*, as these are characteristic species of weakly buffered conditions and are regarded as acid-sensitive species. The dwarf shrub *Calluna vulgaris* and the grasses *D. flexuosa* and *N. stricta* can occur in eutrophied and acidified heathlands and are therefore thought to be acid-tolerant as well as tolerant to high NH_4^+/NO_3^- ratios. We also hypothesised

that lime addition would reduce the negative effects of high NH_4^+/NO_3^- ratios on the growth and survival of herbaceous species, as acidification would be diminished and nitrification would be increased, thus increasing NH_4^+/NO_3^- ratios in the soil.

MATERIALS AND METHODS

Experimental design and plant species

In October 2000, mineral sand (12 m^3) was collected from a dry heathland at the De Hamert nature reserve in the south of the Netherlands (51°32'N, 6°11'E). Sand was collected from a depth of 0 cm down to approx. 30 cm, after removal of the plant material and the organic top layer. The heathland was relatively poor in species, was dominated by *Calluna vulgaris* and suffered from encroachment by grasses, mainly *M. caerulea*. Total annual atmospheric N deposition was estimated to reach, on average, 35 kg ha^{-1} (Ferens *et al.*, 2001). In a greenhouse, 38 polyethylene containers (1.0 x 1.0 x 0.4 m), equipped with drainage holes, were filled with this sand after sieving (mesh size 5 mm) to exclude large particles and organic material. The greenhouse was not heated in order to mimic natural seasonal variance in temperature and humidity (average temperature was 15°C and average air humidity was 75%). The temperature in the greenhouse was ~3.3°C higher than outside. The background dry N deposition in the greenhouse was ~1 kg N ha^{-1} yr^{-1}.
The mesocosms were pre-treated for a period of approx. 10 months with demineralized water (total precipitation 800 mm) to remove the surplus NO_3^- and NH_4^+ from the soil, which had resulted from increased mineralization and nitrification due to soil disturbance during filling. N additions started in August 2001, when $NO_{3\ (H2O)}^-$ concentrations in the soil had decreased to below 150 µmol kg^{-1} DW. Different NH_4^+/NO_3^- ratios in the soil were created at high total N deposition (40 and 60 kg N ha^{-1} yr^{-1}). This was done by applying artificial rain in the form of four different watering solutions, with four replicates each. Solution **(A)** contained 825 µmol l^{-1} NH_4^+ and no NO_3^-, solution, **(B)** 441 µmol l^{-1} NH_4^+ and 110 µmol l^{-1} NO_3^-, solution, **(C)** 275 µmol l^{-1} NH_4^+ and 275 µmol l^{-1} NO_3^- and solution **(D)** 110 µmol l^{-1} NH_4^+ and 441 µmol l^{-1} NO_3^-. These treatments reflected atmospheric N deposition ratios of 1:0, 4:1, 1:1 and 1:4, respectively. These ratios correspond with highly N-polluted deposition in the Netherlands in the 1960's (ratio 1:0), at present (ratios 4:1 and 1:1) and N deposition from areas less dominated by agriculture (ratio 1:4), respectively. NH_4^+ was added as ammonium chloride (NH_4Cl) and NO_3 was added as sodium nitrate ($NaNO_3$). The artificial rainwater was supplied twice a week at a rate of 520 mm yr^{-1} using watering cans. In addition to NO_3^- and NH_4^+ the rainwater contained 66 µmol l^{-1} Na^+, 77.5 µmol l^{-1} Cl^-, 7.5 µmol l^{-1} Mg^{2+}, 4 µmol l^{-1} SO_4^{2-}, 1.5 µmol l^{-1} Ca^{2+} and 1.4 µmol l^{-1} K^+. To half of the mesocosms we added 200 g m^{-2} $CaCO_3$, while the other half of the mesocosms remained unlimed.
In September and October 2001 eight plants each of the species *A. dioica*, *G. pneumonanthe*, *T. serpyllum*, *Calluna vulgaris*, *D. flexuosa*, *Nardus stricta and Danthonia decumbens* and four plants of the species *A. montana* were planted in each mesocosm, resulting in a total of 60 plants per mesocosm. An identical plant composition was

used for all mesocosms, using a fixed 1 x 1 m grid with a mesh width of 10 x 10 cm. The precise plant composition was randomly decided prior to planting. Plants of *T. serpyllum*, *Calluna vulgaris*, *D. flexuosa*, *N. stricta* and *D. decumbens* were obtained as young cuttings from local sods (Nijmegen, Heumensoord; 51°47'N, 5°53'E), whereas plants of *A. dioica*, *G. pneumonanthe* and *A. montana* were grown from seeds originating from wild plants, except for the *A. dioica* seeds that were commercially derived (Blauetikett Borntraeger GmbH, Offstein, Germany). All seedlings were 1 year of age when they were planted in the mesocosms. The shoots and roots of *Calluna vulgaris*, *D. flexuosa*, *N. stricta* and *D. decumbens* were cut back to 8 cm height before planting.

Chemical and biochemical analysis, survival and plant performance

The pH of the soil pore water was analysed by placing three pore water samplers (Rhizon SMS-10 cm; Eijkelkamp Agrisearch Equipment, The Netherlands) in each mesocosm at a depth of 10-15 cm. Soil pore water was collected every two months by connecting a vacuum infusion flask (100 ml) to each sampler. The samples were stored at 4°C (for a maximum of 2 weeks) until further analysis. The pH of the pore water was measured with a Radiometer PHM 82 pH meter.

Four soil samples (0-10 cm depth) from each mesocosm were taken, using an auger (diameter 3 cm), before the start of the experiment, after 6 months and at the end of the experiment. Soil samples from each mesocosm were mixed, resulting in four replicates per treatment. A portion of 35 g of fresh soil was mixed with 100 ml bi-distilled water for water-soluble extraction. For salt extractions, 35 g of fresh soil was mixed with 100 ml 0.2 M NaCl solution. Both extractions were performed in 250 ml polyethylene bottles. After 1 hour of shaking at 100 rpm, pH was measured with a Radiometer type PHM 82 pH meter. The samples were then centrifuged for 20 minutes at 11,000 rpm and the supernatants were stored in polyethylene bottles at -18°C until further analysis. NO_3^- and NH_4^+ concentrations in the soil were measured colorimetrically using a Traacs 800+ auto-analyser.

In March 2004, at the end of the experiment, the aboveground parts of all plants were harvested. After washing, the aboveground biomass of the grasses and *Calluna vulgaris* was determined destructively by drying the plant material for 48 hours at 70°C. Due to lack of material and because we wanted to harvest fresh material from the herbaceous species for amino-acid analysis, their biomass was measured non-destructively (number of leaves x length and width of biggest leaf for *A. montana* and *G. pneumonanthe*; number of leaves x length and width of three largest shoots for *A. dioica* and length x width of plant cover area for *T. serpyllum*; hereafter referred to as estimated biomass). This estimated biomass was highly correlated with the actual biomass (Vergeer *et al.*, 2003). The total biomass of the herbaceous species was measured as the sum of the relative biomass of *G. pneumonanthe*, *A. montana*, *A. dioica* and *T. serpyllum*.

Due to the time-consuming methods used for the analysis of the chlorophyll content and the amino-acid content was restricted to *A. montana*, *D. flexuosa* and *G. pneumonanthe*. Their chlorophyll content was measured as the active chlorophyll-a concentration. Chlorophyll was extracted with 80% ethanol at 70°C. After cooling

and centrifugation light absorption was measured at 665 and 750 nm. The amount of active chlorophyll-a was calculated by correcting for the amount of phaeopigments (degraded chlorophyll). Free amino acids were extracted according to Van Dijk and Roelofs (1988). After precolumn derivation with 9-fluorenylmethyl-chloroformate (FMOC-Cl), fluorescence was measured with HPLC (Star 9050 variable wavelength UV-VIS and Star 9070 fluorescence detector; Varian Liquid Chromatography, Palo Alto, USA). Norleucine was used as the internal standard. The amino acids arginine (ARG), asparaginine (ASN), serine, (SER), glutamine (GLN) and glutamic acid (GLU) were measured and expressed as μmol g^{-1} DW.
To analyse nutrient concentrations in the plants, dried plant material (48 hours at 70°C) was ground in liquid nitrogen, after which 100 mg of the ground material was digested in sealed Teflon vessels in a Milestone destruction microwave oven (MLS 1200 mega) with nitric acid and hydrogen peroxide. The plant material was analysed for Ca, Mg and P using an inductively coupled plasma emission spectrophotometer (ICP, Spectroflame Flame VML2). K levels were determined with a flame photometer, using a Technicon I Auto Analyser (Technicon, New York, USA).

Data analysis

Since we were interested in the effects of different NH_4^+/NO_3^- ratios in the soil on heathland vegetation development and species composition, we analysed the data using regressions on the actual NH_4^+/NO_3^- concentrations and ratios in the soil, calculated as $NH_4^+(NaCl)/NO_3^{-}{}_{(H2O)}$. All data were statistically analysed with the SPSS 11.5 package (SPSS Inc., Chicago, USA) after testing for normality. Effects of different artificial rain treatments and of additional liming on NH_4^+ and NO_3^- concentrations in the soil were tested with General Linear Model (GLM) procedures for repeated measures, with the artificial rain treatments and lime addition as fixed factors. This method was chosen to take into account any divergent concentration effects due to accumulation. When effects of lime addition were analysed, this resulted in 1 degree of freedom wereas 3 degrees of freedom were found when different NH_4^+/NO_3^- ratios in the soil were compared. Effects of the actual NH_4^+/NO_3^- ratios in the soil on biomass, mortality, chlorophyll concentration, amino acid content and internal concentrations of Ca, Mg, K, P and N were tested with regression analyses. All variables were tested to see, whether the assumptions of random and normally distributed errors and homoscedasticity were met (Field, 2005). If necessary, transformations were applied. Biomass values were log-transformed and all percentages were transformed using arcsin-square-root transformations.

RESULTS

Soil chemistry

N treatments in deposition directly affected mean NH_4^+ concentrations in the soil, as increasing NH_4^+/NO_3^- ratios in the artificial rain water resulted in increased NH_4^+/NO_3^- ratios in the soil (**table 1**). Significant differences in NH_4^+/NO_3^- ratios

and NH_4^+ and NO_3^- concentrations in the soil were observed between the two highest ratio treatments (A and B) and between these treatments and the low ratio treatments (C and D). No significant differences were observed between the NH_4^+ concentrations in control and limed mesocosms (*d.f.* = 1, F = 4.144, p = 0.055), although clear trends (lower NH_4^+ concentrations in limed mesocosms) were visible for treatments A, B and C. Of the four treatments, B showed a significant decrease in NH_4^+ concentration in the limed mesocosms (*d.f.* = 1, F = 4.676, p = 0.047). A significant NH_4^+/NO_3^- ratio effect in N deposition was observed for mean NO_3^- concentrations in the soil due to higher NO_3^- concentrations in mesocosms treated with high NH_4^+/NO_3^- ratios (*d.f.* = 3, F = 33.034, p = 0.000). Lime addition did not significantly alter NO_3^- concentrations in the mesocosms, which means that lime addition did not markedly change the NH_4^+/NO_3^- ratios in the soil (**table 1**).
Mean soil pH increased significantly with decreasing NH_4^+/NO_3^- ratio in the atmospheric deposition (from A to D) in both the control and limed mesocosms (*d.f.* = 3, F = 22.366, p = 0.000; **table 1**). Lime addition resulted in a slight, but significant, increase in soil pH (*d.f.* = 1, F = 8.260, p = 0.011). Total base cation concentrations (Ca, Mg and K) increased with lime addition (*d.f.* = 1, F = 13.658, p = 0.001; **table 1**). Increasing NH_4^+/NO_3^- ratios, as applied in the different treatments, did not result in significant differences in base cations.

Table 1: Mean NH_4^+ (NaCl extraction), NO_3^- (H_2O extraction) and base cation concentrations (in µmol kg^{-1} DW; ± s.e.m.), NH_4^+/NO_3^- ratios (± s.e.m.) and pH (H_2O extraction; ± s.e.m.) in the soils of the treatments A-D (see Materials and Methods) in 2003.

	NH_4^+/NO_3^-		NH_4^+		NO_3^-	
	Control	Lime	Control	Lime	Control	Lime
A	2.5 ± 0.4	1.5 ± 0.5	758.5 ± 120.6	555.1 ± 82.3	301.4 ± 18.4	261.7 ± 4.5
B	1.1 ± 0.3	0.6 ± 0.4	223.4 ± 32.8	105.6 ± 14.0	197.5 ± 40.0	164.4 ± 45.2
C	0.7 ± 0.2	0.3 ± 0.1	83.8 ± 10.5	63.8 ± 2.9	112.2 ± 30.4	238.6 ± 48.1
D	0.8 ± 0.1	0.9 ± 0.3	85.9 ± 6.3	93.6 ± 22.7	119.8 ± 24.7	121.4 ± 16.7

[continued]

	pH		Base cations	
	Control	Lime	Control	Lime
A	3.1 ± 0.1	3.2 ± 0.0	5178.6 ± 850.2	10885.4 ± 2451.2
B	3.4 ± 0.1	3.8 ± 0.3	5046.6 ± 668.8	9491.3 ± 1785.6
C	3.9 ± 0.1	4.3 ± 0.2	6911.0 ± 1381.1	7687.5 ± 1056.6
D	4.3 ± 0.1	4.6 ± 0.1	7465.8 ± 377.1	10901.8 ± 1391.0

Plant responses

Biomass

In the control situation the biomass values of the grass *D. decumbens*, the herbaceous species *A. dioica* and the shrub *Calluna vulgaris* were significantly correlated with the NH_4^+/NO_3^- ratio in the soil (**table 2; figures 1a, c**). A similar correlation, at the α = 0.1

level, was found for *A. montana* (**table 2; figure 1a**). Lime addition resulted in a significant increase in biomass of *A. dioica*, *D. decumbens*, *N. stricta* (*d.f.* = 1, *F* = 11.606, *p* = 0.003; *d.f.* = 1, *F* = 8.068, *p* = 0.010, *d.f.* = 1, *F* = 4.810, *p* = 0.040, respectively) and *G. pneumonanthe* at the α = 0.1 level (*d.f.* = 1, *F* = 3.667, *p* = 0.069; data not shown). No significant correlations were found between the biomass and NH_4^+/NO_3^- ratios in the soil for plants grown in the limed mesocosms (**figures 1b, d; table 2**).

Mortality

No mortality was found for the grass *D. flexuosa* or the shrub *Calluna vulgaris* in the control plots and low mortality percentages of at most 10% were found for *N. stricta* (**table 2; figure 2c**). In contrast, high mortality (up to 100%) was found in these mesocosms for the grass *D. decumbens* and the herbaceous species *A. dioica* and *A. montana* (**figures 2a, 2c**). In the control mesocosms significant positive correlations between NH_4^+/NO_3^- ratios in the soil and mortality were found for *D. decumbens*, *A. dioica*, *A. montana* and *G. pneumonanthe* (**figures 2a, c; table 2**). Lime addition significantly reduced mortality of *A. dioica* and, at the α = 0.1 level, of *A. montana* and *G. pneumonanthe*, as was indicated by a GLM analysis (*d.f.* = 1, *F* = 13.331, *p* = 0.001; *d.f.* = 1, *F* = 3.979, *p* = 0.060; *d.f.* = 1, *F* = 3.090, *p* = 0.093, respectively). No significant relations were found for *T. serpyllum*. The positive effect of lime addition on the survival of the other species was reflected in the regression analysis. In the limed mesocosms, only *D. decumbens* showed a significant positive correlation between NH_4^+/NO_3^- ratios in the soil and mortality (**table 2; figure 2d**). No dead *Calluna vulgaris* plants were recorded in the limed mesocosms during the experiment (**table 2**).

Amino acids

Amino acids and nutrient concentrations were measured for the herbaceous species *G. pneumonanthe*, *A. montana* and the grass *D. flexuosa*. For *D. flexuosa* and *G. pneumonanthe* a significant positive correlation was found between the NH_4^+/NO_3^- ratios in the soil of the control plots and the arginine and asparaginine concentrations (**table 3**). For *D. flexuosa* and *G. pneumonanthe*, serine concentrations were found to be significantly correlated (although only at the α = 0.1 level) with NH_4^+/NO_3^- ratios in the soil, with increasing serine concentrations in plants grown at high ratios (**table 3**). *A. montana* plants did not show any significant correlations for arginine or asparaginine in the control mesocosms, but instead showed clear positive correlations between NH_4^+/NO_3^- ratios in the soil and glutamine and serine concentrations. Significant positive correlations were found between NH_4^+/NO_3^- ratios in the soil and the arginine, asparaginine and glutamine concentrations in the plant after lime addition.

In the limed mesocosms the only significant correlations were those between NH_4^+/NO_3^- ratios in the soil and asparaginine and serine concentrations in *D. flexuosa* (**table 3**). There were no significant correlations for *G. pneumonanthe* in the limed mesocosms between NH_4^+/NO_3^- ratios in the soil amino acid concentrations (**table 3**), which was reflected by lower total amino acid concentrations in *G. pneumonanthe* plants grown in the limed mesocosms (385.8 µmol l^{-1}) compared to those grown in the control mesocosms (662.6 µmol l^{-1}, *d.f.* = 1, *F* = 5.768, *p* = 0.026).

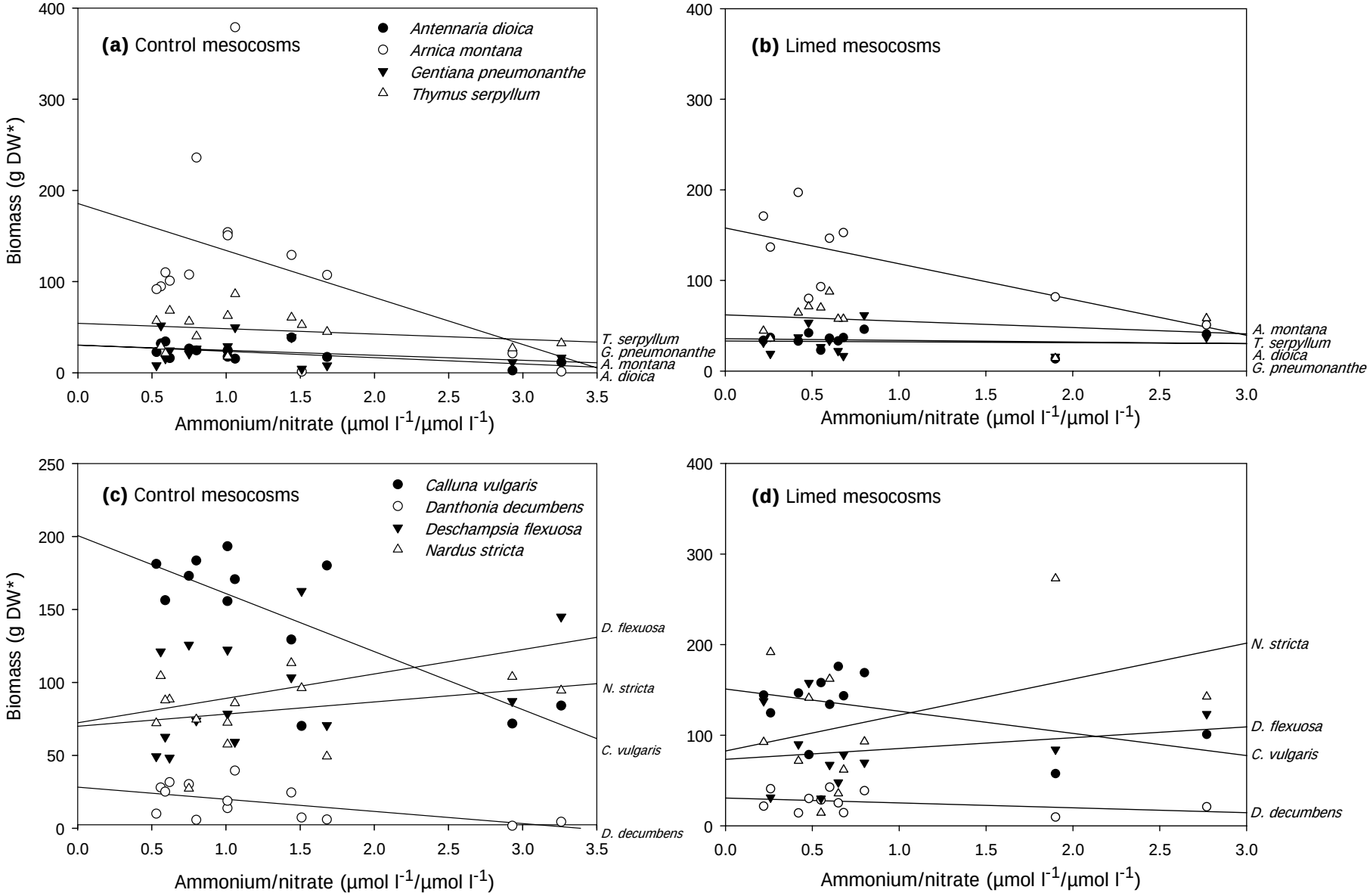

Figure 1: Mean biomass values per plant species in relation to the ammonium/nitrate ratios in the control mesocosms **(a, c)** and limed mesocosms **(b, d)**. Biomass values of *Calluna vulgaris*, *Deschampsia flexuosa*, *Danthonia decumbens*, and *Nardus stricta* are given in g DW; * indicates that the biomass of *Arnica montana* and *Antennaria dioica* is shown as the relative biomass (= no. of leaves * length and width of the longest leaf/100, in mm²). Biomass values of *T. serpyllum* and *Gentiana pneumonanthe* are not shown.

Table 2: Results of regression analysis with the mean ammonium/nitrate ratio per mesocosm as independent and mean final biomass **(a)** or mortality **(b)** as dependant variable. Biomass of the species *Arnica montana*, *Antennaria dioica*, *Gentiana pneumonanthe* and *Thymus serpyllum* were estimated. Biomass of the species *Deschampsia flexuosa*, *Nardus stricta*, *Danthonia decumbens* and *Calluna vulgaris* was measured destructively (in g). *P*-values below $p < 0.05$ are indicated in bold, * indicates $p < 0.1$ and *sl* = direction of the correlation, either positive **(+)** or negative **(-)**.

		(a) Biomass				**(b)** Mortality			
		r^2	*sl*	*F*	*p*	r^2	*sl*	*F*	*p*
Control	*Antennaria dioica*	0.394	-	7.154	0.022	0.405	+	8.174	**0.014**
	Arnica montana	0.207	-	3.132	0.099*	0.614	+	19.291	**0.001**
	Calluna vulgaris	0.585	-	14.089	0.004	*no mortality*			
	Danthonia decumbens	0.342	-	6.25	0.028	0.717	+	30.417	**0.000**
	Deschampsia flexuosa	0.154	+	2.179	0.166	*no mortality*			
	Gentiana pneumonanthe	0.025	-	1.339	0.270	0.377	+	7.249	**0.020**
	Nardus stricta	0.091	+	1.202	0.294	0.025	-	0.303	0.592
	Thymus serpyllum	0.064	-	0.827	0.381	0.042	-	0.525	0.482
Lime	*Arnica montana*	0.023	-	0.207	0.660	0.150	+	2.124	0.171
	Antennaria dioica	0.013	-	1.153	0.306	0.184	+	2.699	0.126
	Calluna vulgaris	0.002	+	0.026	0.875	*no mortality*			
	Danthonia decumbens	0.141	-	1.968	0.186	0.293	+	4.977	**0.046**
	Deschampsia flexuosa	0.017	+	0.212	0.653	0.082	+	0.890	0.368
	Gentiana pneumonanthe	0.013	+	0.149	0.707	0.008	+	0.091	0.768
	Nardus stricta	0.031	+	0.387	0.545	0.000	-	0.001	0.982
	Thymus serpyllum	0.154	-	1.821	0.207	0.088	+	1.064	0.325

Arginine concentrations differed between species and were markedly higher in *A. montana* (74.2 µmol l^{-1}, compared to 11.9 and 1.9 µmol l^{-1} for *G. pneumonanthe* and *D. flexuosa*, respectively). In contrast, asparaginine concentrations in all mesocosms were much higher in *G. pneumonanthe* and *D. flexuosa* than in *A. montana* (data not shown).

Nutrient concentrations

Analysis of the nutrient concentrations in the herbaceous species *G. pneumonanthe* and *A. montana* in the control mesocosms yielded a significant negative correlation between the NH_4^+/NO_3^- ratios in the soil and Mg concentrations (**table 3**). For *G. pneumonanthe* we also found a negative correlation between the NH_4^+/NO_3^- ratios in the soil and the base cation concentrations. A positive correlation between these ratios in the soil and P concentrations was observed for all three species in the control mesocosms, whereas in the limed mesocosms positive correlations between the NH_4^+/NO_3^- ratios in the soil and P concentrations were only observed for *G. pneumonanthe* and *A. montana*. In the control mesocosms, a positive correlation was found between these ratios in the soil and Ca concentrations in *A. montana*, although only at the $\alpha = 0.1$ level. In contrast, a negative correlation was observed between the NH_4^+/NO_3^- ratios in the soil and Ca concentrations in *G. pneumonanthe* (at the $\alpha = 0.1$ level; **table 3**). In the limed mesocosms, *D. flexuosa* plants showed a clear positive correlation between the NH_4^+/NO_3^- ratios in the soil and K and base cation concentrations. These correlations were not observed in the control mesocosms. Mean concentrations of nutrients in the plants are shown in **table 4**. In general, total base cation concentrations increased upon lime addition for all plant species (**table 4**). P concentrations were low in all species and were lowest in *D. flexuosa*, where they did not rise above 15 µmol g^{-1} DW. P concentrations in *A. montana* ranged from 18 to 29 µmol g^{-1} DW, whereas P concentrations in *G. pneumonanthe* ranged from 9 to 19 µmol g^{-1} DW. Ca concentrations differed markedly between species.

Chlorophyll

No significant correlations were found between NH_4^+/NO_3^- ratios in the soil and chlorophyll concentrations in the leaves of the three species. There was, however, a slight negative trend (although not significant) between NH_4^+/NO_3^- ratios in the soil and chlorophyll concentrations in *A. montana*, in that plants grown at high ratios tended to have lower chlorophyll concentrations than those grown at low ratios (0.75 mg g^{-1} DW compared to 0.90 mg g^{-1} DW; $d.f. = 2$, $F = 2.338$, $p = 0.127$; data not shown).

Table 3: Results of regression analysis with mean ammonium/nitrate ratio per mesocosm as independent and amino acid concentration **(a)** and nutrient concentrations **(b)** as dependant variable for the species *Deschampsia flexuosa*, *Arnica montana* and *Gentiana pneumonanthe*. *P*-values below p < 0.05 are indicated in bold, * indicates *p* < 0.1 and *sl* = direction of the correlation, either positive **(+)** or negative **(-),** *b.c.* = base cations.

	(a) Amino acids									**(b)** Nutrient analysis							
	Control				Lime					Control				Lime			
	r^2	*sl*	*F*	*p*	r^2	*sl*	*F*	*p*		r^2	*sl*	*F*	*p*	r^2	*sl*	*F*	*p*
Deschampsia flexuosa																	
arg	0.689	+	27.796	**0.000**	0.246	+	3.910	0.071*	*Ca*	0.017	+	0.205	0.659	0.090	+	0.994	0.342
asn	0.467	+	10.532	**0.007**	0.416	+	8.561	**0.013**	*Mg*	0.079	+	1.023	0.332	0.174	+	2.101	0.178
gln	0.069	-	0.883	0.336	0.038	-	0.471	0.505	*P*	0.508	+	12.378	**0.004**	0.089	+	0.973	0.347
ser	0.550	+	14.672	**0.002**	0.446	+	9.646	**0.009**	*K*	0.121	+	1.233	0.296	0.497	+	9.899	**0.010**
glu	0.051	-	0.639	0.440	0.009	-	0.113	0.742	*b.c.*	0.018	-	0.215	0.651	0.364	+	5.718	**0.038**
Arnica montana																	
arg	0.362	+	3.397	0.115	0.425	+	5.911	**0.041**	*Ca*	0.234	+	3.662	0.080*	0.180	+	2.195	0.169
asn	0.003	+	0.023	0.884	0.507	+	9.242	**0.014**	*Mg*	0.413	-	8.440	**0.013**	0.136	-	1.568	0.239
gln	0.472	+	6.252	**0.041**	0.375	+	5.405	**0.045**	*P*	0.464	+	10.377	**0.007**	0.584	+	14.015	**0.004**
ser	0.743	+	20.257	**0.003**	0.007	-	0.062	0.808	*K*	0.063	-	0.813	0.385	0.066	+	0.701	0.422
glu	0.167	+	1.402	0.275	0.041	+	0.386	0.550	*b.c*	0.000	+	0.000	0.984	0.042	+	0.441	0.522
Gentiana pneumonanthe																	
arg	0.521	+	13.041	**0.004**	0.177	+	2.144	0.174	*Ca*	0.257	-	4.152	0.064*	0.007	-	0.067	0.801
asn	0.556	+	15.022	**0.002**	0.069	+	0.895	0.363	*Mg*	0.341	-	6.201	**0.028**	0.007	-	0.066	0.802
gln	0.560	-	0.716	0.414	0.087	-	1.142	0.306	*P*	0.606	+	18.43	**0.001**	0.415	+	7.086	**0.024**
ser	0.212	+	3.219	0.098*	0.003	+	0.040	0.846	*K*	0.095	-	1.259	0.284	0.000	-	0.004	0.948
glu	0.036	-	0.449	0.515	0.187	-	2.756	0.123	*b.c.*	0.541	-	14.117	**0.003**	0.178	-	2.167	0.172

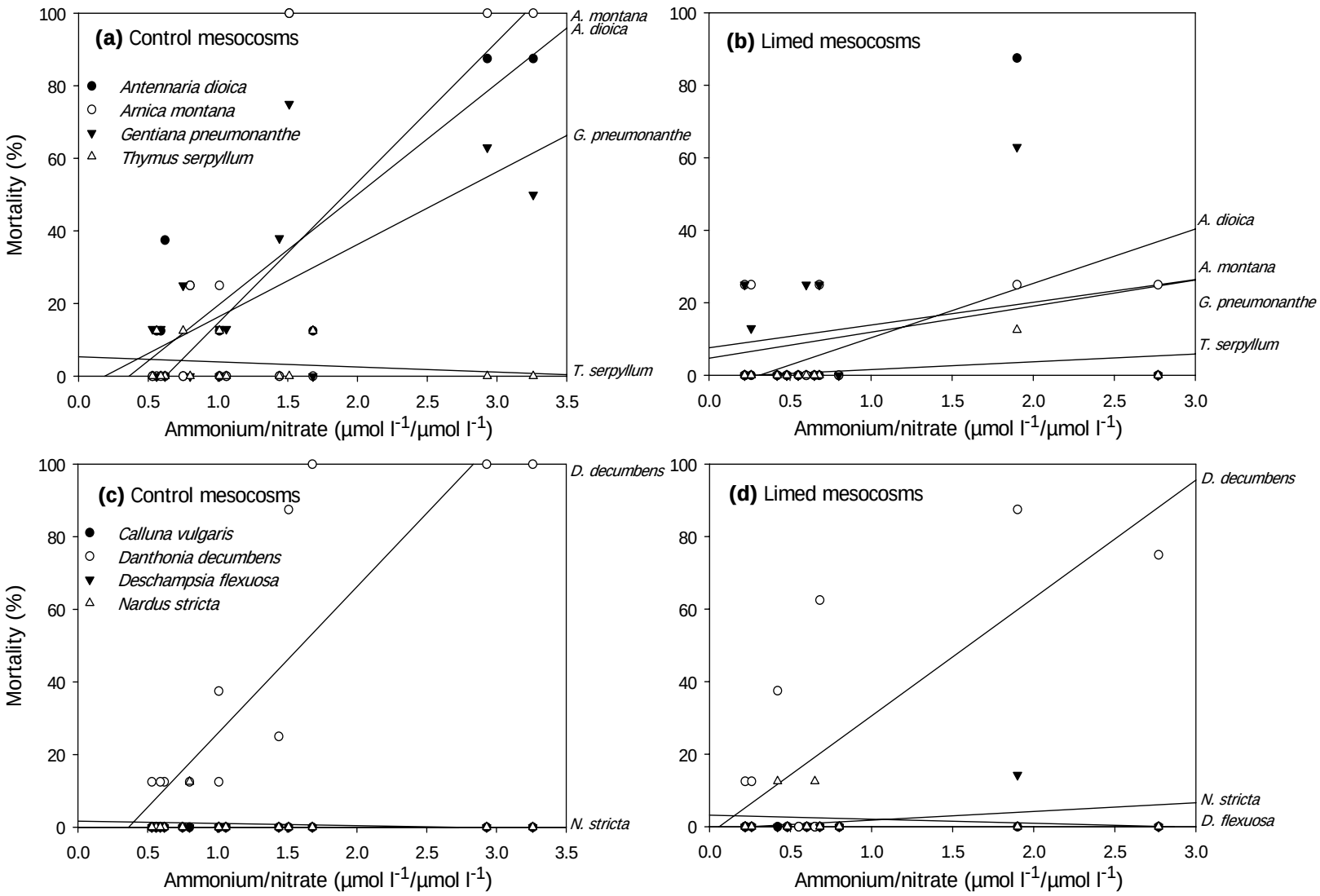

Figure 2: Mean mortality per plant species in relation to the ammonium/nitrate ratio in the control mesocosms **(a, c)** and limed mesocosms **(b, d)**. Mortality is given as percentages (%). No mortality was found for *Calluna vulgaris* and *Deschampsia flexuosa*.

DISCUSSION

Atmospheric N deposition has been suggested to represent a major threat to species-rich heathlands and mat grass swards in Western Europe (see, e.g., Heil and Diemont, 1983; Bobbink *et al.*, 1998). In this study we showed that it is not only the total level of N deposition which can markedly affect the composition of heathland vegetation, but also the form of N and the NH_4^+/NO_3^- ratios in the atmospheric deposition and the soil.

The concentrations of NH_4^+ and NO_3^- that we measured did not accurately reflect the amounts in which these forms of N had been applied. In general, the measured ratios in the soils were lower than the applied ratios. Comparable differences were also observed in a mesocosm study with Scots pine plants by Arnold (1992), who attributed this to a high NH_4^+ uptake by plants. Arnold (1992) found that NH_4^+ uptake always exceeded NO_3^- uptake irrespective of the dominant N source. NH_4^+ uptake may also have been responsible for a decrease in NH_4^+ concentrations in our mesocosms. In addition, some of the applied NH_4^+ may have been absorbed onto exchange sites in organic material, as the percentage organic material ranged from 10% to 12%. However, a large part of the NH_4^+ may have been converted into NO_3^- (nitrification), as was indicated by the increased NO_3^- concentrations at elevated NH_4^+ concentrations. This effect was most apparent in the lime-treated mesocosms in which the NH_4^+ concentrations and hence NH_4^+/NO_3^- ratios, decreased significantly in the A, B and C treatments ($p = 0.008$, $F = 10.326$, *d.f.* = 1; **table 1**). This supports the idea that there was a relatively high level of nitrification. Nitrification has indeed been shown to be low at low pH and to increase at higher pH levels (Roelofs *et al.*, 1985; De Boer *et al.*, 1988). In the control mesocosms pH increased over time in all treatments, which we attributed to NO_3^- uptake by the plants. Since the measured and applied NH_4^+ and NO_3^- concentrations did not correspond, we analysed the effects of NH_4^+/NO_3^- ratios on plant growth, performance and nutrients using regression analysis with the actual ratios measured in the soil.

The responses of the plants to different NH_4^+/NO_3^- ratios in the soil were variable. In general, the herbaceous species tended to show a negative correlation between NH_4^+/NO_3^- ratios in the soil and biomass production, whereas grasses such as *N. stricta* and *D. flexuosa* showed no correlation with NH_4^+/NO_3^- ratios in the soil, or a slight (non-significant) positive correlation. The herbaceous species *A. dioica*, *G. pneumonanthe* and *A. montana* and the grass *D. decumbens* proved to be sensitive to high NH_4^+/NO_3^- ratios, as their mortality increased with increasing ratios in the control mesocosms.

A recent study using hydroponics showed that *A. dioica* was sensitive to high NH_4^+ concentrations (> 500 µmol l^{-1}) in combination with a low (3.5) pH (**chapter 3**). In contrast, *G. pneumonanthe* proved insensitive to elevated NH_4^+ concentrations and acid conditions (**chapter 3**). The present results are in partial agreement with that study, as is illustrated by the increased survival and biomass after lime addition. However, since there was a relatively high level of nitrification, NH_4^+ concentrations did not increase to toxic levels (> 500 µmol l^{-1}), so no clear interaction effect was found between high NH_4^+ concentrations and acidification. The decline of *A. dioica* in our mesocosms was therefore probably mainly a result of the acid soil conditions.

Table 4: Mean concentrations of Ca, Mg, P, K and base cations (in µmol g^{-1} DW) in shoots of the species *Arnica montana*, *Deschampsia flexuosa* and *Gentiana pneumonanthe* of the different treatments (A-D). Mean values (± s.e.m.) for plants from control and limed mesocosms are shown.

	A		*B*		*C*		*D*	
	Control	Lime	Control	Lime	Control	Lime	Control	Lime
Arnica montana								
Ca	353.9 ± 23.0	427.0 ± 56.3	335.4 ± 16.4	312.6 ± 15.9	286.4 ± 13.5	315.5 ± 13.6	305.5 ± 19.2	302.5 ± 20.9
Mg	79.3 ± 2.2	86.9 ± 8.3	103.1 ± 5.0	94.9 ± 7.3	91.8 ± 4.2	103.7 ± 7.9	112.4 ± 2.4	108.9 ± 11.6
P	28.4 ± 0.6	36.4 ± 2.8	24.5 ± 4.3	23.5 ± 0.7	22.1 ± 2.3	22.6 ± 2.8	20.4 ± 1.9	20.8 ± 2.2
K	17.3 ± 1.6	58.3 ± 35.7	35.1 ± 6.9	57.7 ± 11.0	57.8 ± 20.9	65.5 ± 15.1	39.1 ± 6.0	41.3 ± 3.7
Base cations	477.9 ± 25.7	605.5 ± 51.4	501.2 ± 2.8	497.8 ± 27.2	479.3 ± 35.9	535.4 ± 24.8	502.5 ± 11.5	495.0 ± 36.4
Deschampsia flexuosa								
Ca	172.7 ± 35.8	173.1 ± 39.4	138.5 ± 16.2	175.1 ± 11.3	111.4 ± 17.8	140.3 ± 18.7	138.5 ± 20.5	133.0 ± 10.5
Mg	158.5 ± 40.8	118.9 ± 21.2	103.4 ± 8.1	92.7 ± 5.7	69.9 ± 8.9	91.0 ± 9.4	95.0 ± 10.3	96.3 ± 5.3
P	30.4 ± 3.2	22.1 ± 7.4	9.6 ± 2.7	12.1 ± 2.6	8.3 ± 1.3	10.9 ± 1.7	7.5 ± 0.3	13.0 ± 3.5
K	50.4 ± 14.2	182.9 ± 85.2	76.9 ± 8.1	86.5 ± 30.5	50.1 ± 17.4	63.4 ± 18.8	51.0 ± 12.4	59.9 ± 14.1
Base cations	340.8 ± 76.9	436.8 ± 116.7	337.7 ± 39.4	375.6 ± 43.6	274.8 ± 51.6	331.6 ± 27.6	330.8 ± 36.8	325.1 ± 13.6
Gentiana pneumonanthe								
Ca	78.2 ± 4.4	153.8 ± 11.7	141.8 ± 10.6	149.2 ± 7.5	118.6 ± 8.9	154.5 ± 5.7	102.3 ± 5.0	104.8 ± 1.9
Mg	39.8 ± 3.6	102.1 ± 3.2	99.6 ± 5.3	99.6 ± 4.0	87.5 ± 4.1	110.7 ± 3.6	76.5 ± 0.8	79.79 ± 1.7
P	29.8 ± 4.8	28.6 ± 3.9	15.1 ± 0.9	18.7 ± 1.1	14.7 ± 0.6	9.7 ± 0.6	12.2 ± 0.8	9.80 ± 0.5
K	37.3 ± 7.2	113.9 ± 28.6	78.4 ± 4.4	108.4 ± 4.8	96.7 ± 4.0	107.2 ± 4.2	54.7 ± 1.6	30.37 ± 2.8
Base cations	171.4 ± 11.3	396.1 ± 30.1	392.0 ± 23.4	450.7 ± 17.7	415.2 ± 24.5	518.1 ± 28.2	408.5 ± 2.9	391.00 ± 7.7

A. montana has been shown to be highly sensitive to increased Al/Ca ratios (De Graaf *et al.*, 1997). Al concentrations increase at lower pH levels, as a result of a shift in soil buffer range (Scheffer and Schachtschabel, 1979). It has been suggested that the decline of key species such as *A. montana* is due to these high Al concentrations, which cause severe Al toxicity, rather than to the effects of NH_4^+ toxicity (De Graaf *et al.*, 1997, **chapter 6**). This may also apply to *G. pneumonanthe*. We measured low Al concentrations (on average 58 μmol kg^{-1} DW) and low Al/Ca ratios (Al/Ca < 1). The absence of high Al/Ca ratios might explain the fact that neither *A. montana* nor *G. pneumonanthe* showed negative relations with NH_4^+/NO_3^- ratios in the soil.
Earlier mesocosm experiments have shown that *T. serpyllum* is sensitive to high NH_4^+ concentrations, as its cation uptake is greatly reduced by NH_4^+ uptake (Houdijk *et al.*, 1993). However when nitrification rates were increased, NO_3^- concentrations compensated for high NH_4^+ concentrations, resulting in sufficient cation uptake (Houdijk *et al.*, 1993). The fact that we found low mortality of *T. serpyllum* in all mesocosms may suggest that nitrification rates and/or NO_3^- concentrations were relatively high in all mesocosms, preventing cation deficiency in this species. *D. decumbens,* a characteristic slow-growing grass of species-rich and nutrient-poor grasslands (Swertz *et al.*, 1996; Glimskär, 2000), proved sensitive to the high NH_4^+/NO_3^- ratios in the soil. Lime addition improved the survival and biomass production of this grass, suggesting that the species is acid-sensitive. In addition, it may also be sensitive to increased NH_4^+/NO_3^- ratios, as NO_3^- concentrations in the limed mesocosms were considerable higher due to nitrification. A decline in biomass at high NH_4^+/NO_3^- ratios was also observed for the shrub *Calluna vulgaris*, illustrating that it was affected by high NH_4^+/NO_3^- ratios and/or high NH_4^+ concentrations. These results are corroborated by a hydroponic experiment, which showed that the growth of *Calluna vulgaris* was reduced and indications of chlorosis were apparent when plants were grown at high (1000 μmol l^{-1}) NH_4^+ concentrations and low (3.5) pH (**chapter 3**).
The herbaceous species *A. montana* and *G. pneumonanthe* and the grass *D. flexuosa* showed similar responses in terms of the amino acid production and nutrient composition to different NH_4^+/NO_3^- ratios in the soil. All three species showed increased concentrations of arginine and/or asparaginine when grown at high NH_4^+/NO_3^- ratios. Such an increase in free amino acid concentrations at increased NH_4^+ uptake has also been found in other studies (see, e.g., Van Dijk and Roelofs, 1988; Thomassen *et al.*, 2003). To prevent NH_4^+ toxicity many plants respond to NH_4^+ uptake by synthesising specific amino acids and amines (Marschner, 1995). In particular they produce amino acids with a low C/N ratio such as arginine (C/N ratio = 6/4) and asparaginine (4/2; Marschner, 1995). Only in the concentrations of base cations were some differences between species visible. *D. flexuosa* and, for some elements (Ca concentrations and total base cation concentrations), *A. montana* showed a positive correlation with NH_4^+/NO_3^- ratios in the soil. In contrast, *G. pneumonanthe* showed a negative correlation, indicating that increased NH_4^+/NO_3^- ratios may eventually lead to cation deficiency for this species. However, as mortality was low and base cation concentrations were not low, we found no base cation deficiency in our plants. The positive correlation between base cation concentrations

and NH_4^+/NO_3^- ratios in the soil for *A. montana* was due to the strong positive correlation with Ca; all other base cations showed a negative correlation. Hence, if we ignore Ca, the correlations for Mg^{2+} and K^+ also point in the direction of base cation deficiency with increasing NH_4^+/NO_3^- ratios in the soil. Reduced cation uptake and even cation deficiency upon NH_4^+ nutrition, has also been found in other studies (Boxman *et al.*, 1991; Pearson and Stewart, 1993). Moreover, Mg^{2+} and K^+ deficiency, rather than Ca^{2+} deficiency, was found when *A. montana* was grown at elevated NH_4^+ concentrations (De Graaf *et al.*, 1998).

Implications for heathland vegetation

The results of this study suggest that both grass species and herbaceous species take up NH_4^+. The ability to detoxify NH_4^+ and to withstand acidified soil conditions thus becomes decisive for the presence or absence of species, especially when these are grown at high NH_4^+/NO_3^- ratios. It is clear from the results that most herbaceous species and the grass *D. decumbens* prefer low NH_4^+/NO_3^- ratios. *A. montana*, *G. pneumonanthe*, *D. decumbens* and *Calluna vulgaris* all showed characteristic symptoms of a NH_4^+ toxicity syndrome (Mehrer and Mohr, 1989), indicated by yellow leaves and stunted roots (data not shown). For *Calluna vulgaris*, this was also shown by the study described in **chapter 3** and indications of poor growth at 1000 µmol l^{-1} NH_4^+ were also found in a study by De Graaf *et al.* (1998), although the effects were not significant.

In the field, species-rich mat grass swards and heathlands, characterised by the endangered species *A. montana*, *A. dioica*, *G. pneumonanthe* and *T. serpyllum* generally occur on soils with a slightly higher soil pH and higher concentrations of base cations, than species-poor grasslands and degraded heathlands. This was also found in a hydroponic experiment performed by Van Dobben (1991), which illustrated that *A. montana*, *A. dioica* and *G. pneumonanthe* had a higher pH optimum (4.2-6.0), than the supposed acid-tolerant grasses such as *D. flexuosa* (pH optimum 4.0). However, some herbaceous species (*A. montana*, *G. pneumonanthe*) were found to be able to survive very low pH levels without damage in these experiments (Van Dobben, 1991). *A. dioica* is considered to be an acid-sensitive herbaceous species, the decline of which has been attributed in the Netherlands to factors as acidification and a poor ability to establish in suitable habitats (Van Dam *et al.*, 1986; Weeda *et al.*, 2003).

Our results support the hypothesis that acidification and high NH_4^+/NO_3^- ratios negatively affect the survival and fitness of the herbaceous species *A. dioica*, *A. montana* and *G. pneumonanthe* and the grass *D. decumbens*. In contrast, acid-tolerant grasses such as *D. flexuosa* and *N. stricta* are not negatively affected by high NH_4^+/NO_3^- ratios. As was shown, these grasses can effectively detoxify NH_4^+ and thereby use NH_4^+ as their mineral form of N. In addition to the direct toxicity effects at elevated NH_4^+/NO_3^- ratios in the soil it is also competition with fast growing species such as the grass *D. flexuosa*, which explains the decline of many acid-sensitive and NH_4^+-sensitive species in heathlands in Western Europe and their replacement by these competitive grasses.

It has been demonstrated that *D. flexuosa* is more competitive than the slow-growing *Calluna vulgaris* (Heil and Diemont, 1983; Roelofs, 1986; Aerts *et al.*, 1990) but only starts to dominate in heathlands when the canopy of *Calluna vulgaris* is opened up by processes such as frost damage and herbivory by heather beetle (*Lochmaea suturalis*; Aerts and Heil, 1993; Marrs, 1993; Bobbink *et al.*, 1998; Power *et al.*, 1998). Since the results of our study show that *Calluna vulgaris* is sensitive to high NH_4^+/NO_3^- ratios in acidified soils, we suggest that that grasses may well start to dominate dwarf shrub dominated heathlands as a result of a direct decline in *Calluna vulgaris* due to elevated NH_4^+/NO_3^- ratios or NH_4^+ concentrations (**figure 3**).
As the decline of species in poorly buffered systems has been shown to be correlated to severe acidification as a result of nitrification (Schuurkens *et al.*, 1987; De Graaf *et al.*, 1998) and since this effect depends on the actual availability of ammonium, an interaction between increased NH_4^+/NO_3^- ratios and acidification is very likely.

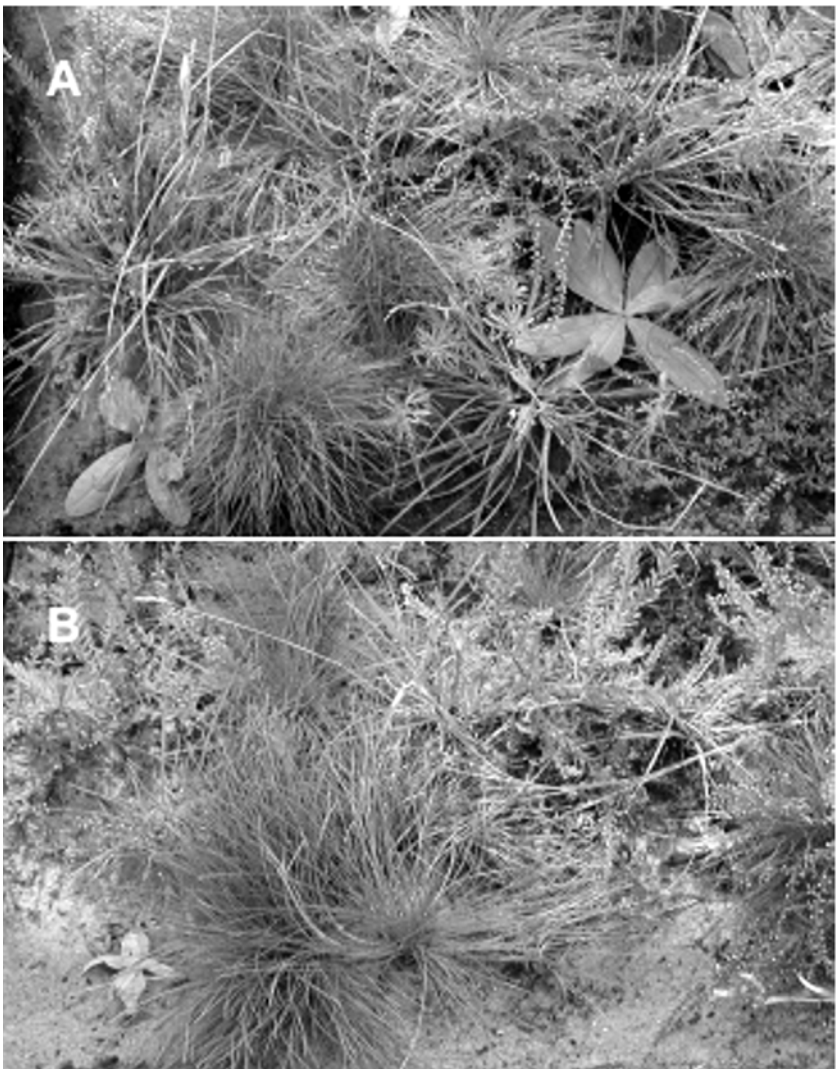

Figure 3: Plants in mesocosms with low **(a)** and high **(b)** NH_4^+/NO_3^- ratios in the soil, after two years. In mesocosm **(b)**, many of the herbaceous species have disappeared (e.g. *Antennaria dioica*) or declined (*Arnica montana*).

We found that atmospheric N deposition and particularly the form in which N is deposited, affects the ecosystems drastically and reduced N (elevated NH_4^+/NO_3^- ratios) was a strong determining factor in vegetation composition. This is an important fact to keep in mind in policy and nature management decisions, as it is of great importance to know the mechanisms and impact on the vegetation composition of the different forms of N and their interactions.

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REFERENCES

Aerts, R., Berendse, F., De Caluwe, H., Schmitz, M. 1990. Competition in heathland along an experimental gradient of nutrient availability. *Oikos,* 57: 310-318.

Aerts, R., Heil, G.W. 1993. *Heathlands: patterns and processes in a changing environment.* Kluwer Academic Publishers, Dordrecht, The Netherlands.

Arnold, G. 1992. Soil acidification as caused by the nitrogen uptake pattern of Scots pine (*Pinus sylvestris*). *Plant and Soil*, 142: 41-51.

Bennet, A.C., Adams, F. 1969. Calcium deficiency and ammonia toxicity as separate causal factors of (NH4)2HPO4 injury to seedlings. *Soil Science Society of America Journal*, 34: 225-228.

Bobbink, R., Heil, G.W. 1993. Atmospheric deposition of sulphur and nitrogen in heathland ecosystems. In: Aerts, R., Heil, G.W. (Eds.). *Heathlands: patterns and processes in a changing environment. Geobotany 20.* Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 25-50.

Bobbink, R., Heil, G.W., Raessen, M.B.A.G. 1992. Atmospheric deposition and canopy exchange processes in heathland ecosystems. *Environmental Pollution*, 75: 29-37.

Bobbink, R., Hornung, M., Roelofs, J.G.M. 1998. The effects of air borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, 86: 717-738.

Boxman, A.W., Krabbendam, H., Bellemakers, M.J.S., Roelofs, J.G.M. 1991. Effects of ammonium and aluminium on the development and nutrition of *Pinus nigra* in hydroculture. *Environmental Pollution*, 73: 119-136.

Carroll, J.A., Campbell, C.D., Caporn, S.J.M., Cawley, L., Johnson, D., Leake, J.R., Lee, J.A., Lei, Y., Morecroft, M.D., Read, D.J., Taylor, A. 1997. *Natural vegetation responses to atmospheric nitrogen deposition: critical levels and loads for nitrogen for vegetationgrowing on contrasting soils. Final report May 1994-1997.* Department of the Environment Research. Contract Reference EPG 1/3/11. Department of Animal and Plant Sciences, University of Sheffield, United Kingdom.

Carroll, J.A., Caporn, S.J.M., Johnson, D., Morecroft, M.D. Lee, J.A. 2003. The interactions between plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition. *Environmental Pollution*, 121: 363-376.

De Boer, W., Duyts, H., Laanbroek, H.J. 1988. Autotrophic nitrification in a fertilized acid heath soil. *Soil Biology and Biochemistry*, 20: 845-850.

De Graaf, M.C.C., Bobbink, R., Roelofs, J.G.M., Verbeek, P.J.M. 1998. Differential effects of ammonium and nitrate on 3 heathland species. *Plant Ecology*, 135: 185-196.

De Graaf, M.C.C., Bobbink, R., Verbeek, P.J.M., Roelofs, J.G.M. 1997. Aluminium toxicity and tolerance in three heathland species. *Water, Air and Soil Pollution*, 98: 229-241.

De Smidt, J.T. 1975. *Nederlandse heidevegetaties (Heathland vegetations of the Netherlands)*. PhD Thesis, Utrecht University, Utrecht, The Netherlands.

De Smidt, J.T. 1979. Origin and destruction of Northwest European heath vegetation. In: Wilmanns, O., Tüxen, R. (Eds.). *Werden und Vergehen von Pflanzengesellschaften*. Cramer Vaduz, Liechtenstein, pp. 411-435.

Diekmann, M., Falkengren-Grerup, U. 1998. A new species index for forest vascular plants: development of functional indices based on mineralization rates of various forms of soil nitrogen. *Journal of Ecology*, 86: 269-283.

Diekmann, M., Falkengren-Grerup, U. 2002. Prediction of species response to atmospheric nitrogen deposition by means of ecological measures and life history traits. *Journal of Ecology*, 90: 108-120.

Eerens, H.C., Van Dam, J.D., Beck, J.P., Dolmans, J.H.J., Van Pul, W.A.J., Sluyter, R.B.C., Van Velze, K. , Vissenberg, H.A. 2001. *Grootschalige luchtverontreiniging en depositie in de Nationale Milieuverkenning 5*. RIVM report 408129016/2001. Bilthoven, The Netherlands.

Falkengren-Grerup, U., Schöttelndreier, M. 2004. Vascular plants as indicators of nitrogen enrichment in soil. *Plant Ecology*, 172: 51-62.

Field, A.P. 2005. *Discovering statistics using SPSS* (2nd edition). Sage, London, United Kingdom.

Galloway, J.N. 1995. Acid deposition: perspectives in time and space. *Water, Air and Soil Pollution*, 85: 15-24.

Galloway, J.N., Cowling, E.B. 2002. Reactive nitrogen and the world: 200 years of change. *AMBIO*, 31: 64-71

Gigon, A., Rorison, I.H. 1972. The response of some ecologically distinct plant species to nitrate- and to ammonium-nitrogen. *Journal of Ecology*, 60: 93-102.

Gimingham, C.H., Chapman, S.B., Webb, N.R. 1979. European heathlands. In: Specht, R.L. (Ed.). *Ecosystems of the world, 9A*. Elsevier, Amsterdam, The Netherlands, pp. 365-386.

Glimskär, A. 2000. Estimates of root system topology of five plant species grown at steady-state nutrition. *Plant and Soil*, 227: 249-256.

Heil, G.W., Aerts, R. 1993. General introduction. In: Aerts, R., Heil, G.W. (Eds.). *Heathland: patterns and processes in a changing environment*. Kluwer, Dordrecht, The Netherlands, pp. 1-24.

Heil, G.W., Diemont, W.H. 1983. Raised nutrient levels change heathland into grassland. *Vegetatio*, 53: 113-120.

Houdijk, A.L.F.M., Verbeek, P.J.M., Van Dijk, H.F.G., Roelofs, J.G.M. 1993. Distribution and decline of endangered herbaceous heathland species in relation to the chemical composition of the soil. *Plant and Soil*, 148: 137-143.

Kellner, O., Redbo-Torstensson, P. 1995. Effects of elevated nitrogen deposition on the field-layer vegetation in coniferous forests. *Ecological Bulletins*, 44: 227-237.

Lucassen, E.C.H.E.T., Bobbink, R., Smolders, A.J.P., Van der Ven, P.J.M., Lamers, L.P.M., Roelofs, J.G.M. 2003. Interactive effects of low pH and high ammonium levels responsible for the decline of *Cirsium dissectum* (L.) Hill. *Plant Ecology*, 165: 45-52.

Marrs, R.H. 1993. An assessment of change in Calluna heathlands in Breckland, Eastern England, between 1983 and 1991. *Biological Conservation*, 65: 133-139.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants* (2nd edition). Academic Press, London, United Kingdom.

Mehrer, I., Mohr, H. 1989. Ammonium toxicity: description of the syndrome in *Sinapsis alba*

and the search for its causation. *Physiologia Plantarum*, 77: 545-554.
Morecroft, M.D., Sellers, E.K., Lee, J.A. 1994. An experimental investigation into the effects of atmospheric nitrogen deposition on two semi-natural grasslands. *Journal of Ecology*, 82: 475-483.
Pearson, J., Stewart, G.R. 1993. Tansley Review No 56: The deposition of atmospheric ammonia and its effects on plants. *New Phytologist*, 125: 283-305.
Polle, A., Otter, T., Mehne-Jakobs, B. 1994. Effects of magnesium-deficiency on antioxidative systems in needles of Norway spruce [*Picea abies* (L.) Karst.] grown with different ratios of nitrate and ammonium as nitrogen sources. *New Phytologist*, 128: 621-628.
Power, S.A., Ashmore, M.R., Cousins, D.A., Sheppard, L.J. 1998. Effects of nitrogen addition on the stress sensitivity of *Calluna vulgaris*. *New Phytologist*, 138: 663-673.
Roelofs, J.G.M. 1986. The effect of airborne sulphur and nitrogen deposition on aquatic and terrestrial heathland vegetation. *Experientia*, 42: 372-377.
Roelofs, J.G.M., Bobbink, R., Brouwer, E., De Graaf, M.C.C. 1996. Restoration ecology of aquatic and terrestrial vegetation on non-calcareous sandy soils in the Netherlands. *Acta Botanica Neerlandica*, 45: 517-541.
Roelofs, J.G.M., Kempers, A.J., Houdijk, L.F.M., Jansen, J. 1985. The effect of air-borne ammonium sulphate on *Pinus nigra* var. *maritima* in the Netherlands. *Plant and Soil*, 84: 45-56.
Scheffer, F., Schachtschabel, P. 1979. *Lehrbuch der Bodenkunde*. Ferdinand Enke Verlag, Stuttgart, Germany.
Schuurkens, J.A.A.R., Elbers, M.A., Gudden, J.J.F., Roelofs, J.G.M. 1987. Effects of simulated ammonium sulphate and sulphuric acid rain on acidification, water quality and flora of small-scale soft water systems. *Aquatic Botany*, 28: 199-226.
Smart, S.M., Ashmore, M.R., Hornung, M., Scott, W.A., Fowler, D.A., Dragosits, U., Howard, D.C., Sutton, M.A., Famulari, D. 2004. Detecting the signal of atmospheric N deposition in recent national-scale vegetation change across Britain. *Water, Air and Soil Pollution: Focus*, 4: 296-278.
Swertz, C.A. Schamineé, J.H.J., van Dijk, E. 1996. In: Schamineé, J.H.J., Stortelder, A.H.F., Weeda, E.J. (Eds.). *Plantengemeenschappen van graslanden, zoden en droge heiden. Deel 3. De vegetatie van Nederland*. Opulus Press, Uppsala, Sweden, pp. 263-286.
Thomassen, H.B.M., Smolders, A.J.P., Lamers, L.P.M., Roelofs, J.G.M. 2003. Stimulated growth of *Betula pubescens* and *Molinia caerulea* on ombrotrophic bogs: role of high levels of atmospheric nitrogen deposition. *Journal of Ecology*, 91: 357-370.
Van Dam, D., Van Dobben, H.F., Ter Braak, C.F.J., De Wit, T. 1986. Air pollution as a possible cause for the decline of some phanerogamic species in the Netherlands. *Vegetatio*, 65: 47-52.
Van Dijk, H.F.G., Roelofs, J.G.M. 1988. Effects of excessive ammonium deposition on the nutritional status and conditions of Pine needles. *Physiologia Plantarum*, 73: 494-501.
Van Dobben, H.F. 1991. Integrated effects (low vegetation). In: Heij, G.J., Schneider, T. (Eds.). *Final report of the Dutch priority programme on Acidification*. Elsevier, Amsterdam, The Netherlands, pp. 464-524.
Vergeer, P., Rengelink, R., Copal, A., Ouborg, N.J. 2003. The interacting effects of genetic variation, habitat quality and population size on performance of *Succisa pratensis*. *Journal of Ecology,* 91: 18-26.
Webb, NR. 2002. Atlantic heathlands. In: Perrow, M.R., Davy, A.J. (Eds.). *Handbook of ecological restoration. Volume 2: restoration in practice*. Cambridge University Press, Cambridge, United Kingdom, pp. 401-418.
Weeda, E.J., Westra, R., Westra, C.H., Westra, T. 2003. *Nederlandse oecologische flora, wilde planten en hun relaties. Deel 4*. KNNV publisher, The Netherlands.

Chapter 5

THE EFFECTS OF SOD CUTTING AND ADDITIONAL LIMING ON POTENTIAL NET NITRIFICATION IN HEATHLAND SOILS

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SUMMARY The effects of sod cutting, a common restoration measure to remove excess nutrients from grass-dominated heathlands, on nitrification were studied in dry and wet Dutch heathlands and in incubation experiments. In the field, soil ammonium and nitrate concentrations were measured after treatment by sod cutting, with or without additional liming. Potential net nitrification was measured by incubating soil samples of all treatments with extra ammonium in a climate chamber at pH 6. Potential net nitrification of heaths dominated by *Molinia caerulea* was significantly higher than that of dwarf-shrub dominated heaths. Sod cutting of the former areas significantly decreased potential net nitrification, whereas in the latter areas no differences were found. Liming of sod-cut soils greatly increased potential net nitrification and the accumulation of ammonium in the soil up to toxic concentrations could be prevented. Our results show that the combination of sod cutting and liming would create suitable soil conditions for the germination and establishment of endangered plant species of dry and wet heathlands. The success of restoration projects of these areas can thus be increased.

INTRODUCTION

Restoration of former species-rich dry and wet heathlands and related matgrass swards is of increasing importance in north-western Europe. Soil acidification and eutrophication resulting from atmospheric deposition of sulphur and nitrogen compounds and drainage have led to the decline of many characteristic plant species of these ecosystems (Bobbink *et al.*, 1998b; Houdijk *et al.*, 1993; Roelofs *et al.*, 1996; Roem *et al.*, 2002; Van Breemen *et al.*, 1982). In recent decades, several countries have applied intensive management programmes to preserve the remaining semi-natural areas and to restore the former species-rich communities. The primary management tool is the reintroduction of former management practices such as mowing, grazing and sod cutting (removal of vegetation and topsoil layer). In wet heathlands and matgrass swards restoration of the original hydrology, for example by blocking drainage ditches, is also applied.

In the Netherlands restoration activities have infrequently resulted in the development of species-rich heaths or matgrass swards (Bobbink *et al.*, 1998a; Jansen *et al.*, 1996; Roelofs *et al.*, 1996). The majority of the restored heathlands developed into species-poor communities that did not include the endangered heath species. The lack of success of restoration projects has often been attributed to dispersal limitations of the target species and to the absence of viable seeds of these species in the seed bank (Bakker *et al.*, 1996; Britton *et al.*, 2000; Jansen *et al.*, 1996). Yet, these can be partial explanations, because failure to restore the former species-rich vegetation has also occurred on sites where seeds of the endangered species were present in the seed bank and where populations of these species existed in nearby sites (Bobbink *et al.*, 1998a). Apparently, other constraints also prevent the return and establishment of target species in restored heathlands and matgrass swards.

One of the bottlenecks in the restoration procedure is the cutting of sods. Sod cutting is commonly used as an effective measure to remove excess nutrients (Aerts and Heil, 1993; Bakker, 1989; Mitchell *et al.*, 2000; Snow and Marrs, 1997). It also exposes bare soil which necessary for the successful seed germination and establishment of many heathland species (Britton *et al.*, 2000; De Graaf *et al.*, 1998b). Previous research has demonstrated another consequence of sod cutting of both dry and wet heathlands. Ammonium (NH_4^+) accumulates in the soil for 1 to 2 years (De Graaf *et al.*, 1995; De Graaf *et al.*, 1998b; Dorland *et al.*, 2003) and the NH_4^+ concentrations reach values that are known to be harmful to many endangered target species. Germination and establishment of target species is less at high NH_4^+ concentrations, especially when pH is low (De Graaf *et al.*, 1998a; Lucassen *et al.*, 2003; Van den Berg, personal observation). Consequently, for an initial period following sod cutting, soil conditions are clearly not suitable for successful germination and establishment of NH_4^+-sensitive plant species. Unsuccessful establishment following germination may lead to further depletion of the seed bank. Common species that are more NH_4^+-tolerant such as *Molinia caerulea*, *Erica tetralix*, *Calluna vulgaris and Juncus bulbosus* readily germinate and may dominate the vegetation. Successful restoration of the former species-rich wet and dry heathlands and matgrass swards thus requires measures to prevent or counteract the detrimental effects of increased NH_4^+ following sod cutting.

To avoid an increase of NH_4^+ following sod cutting a better understanding of its causes is necessary. It could be, because either plant uptake of NH_4^+ ceases, or it may be due to atmospheric deposition, because almost 70% of the nitrogen deposition in the Netherlands is in the form of NH_4^+ (Bobbink *et al.*, 1992; Erisman, 1992). Even though atmospheric deposition has slightly decreased recently, present values are still greater than the critical loads proposed for these ecosystems (viz. 10-25 kg N ha^{-1} yr^{-1}; Bobbink *et al.*, 2003). A third cause may be a temporary increase in mineralization after sod cutting. Mineralization might increase because the remaining roots have relatively high nitrogen concentrations and are easily degraded (Berendse, 1990). NH_4^+ accumulation may also result from decreased nitrifying activity after sod cutting. Nitrifying bacteria mainly occur in the topsoil (Troelstra *et al.*, 1990) and sod cutting might reduce nitrification capacity by removing large quantities of nitrifying bacteria together with the sods. Sod cutting may also create soil conditions that are less suitable for nitrification, for example, reduced soil moisture content (Davidson *et al.*, 1993; Krave *et al.*, 2002; Owen *et al.*, 2003). As a consequence, less NH_4^+ will be converted into nitrate (NO_3^-). Roelofs *et al.* (1996) suggested that nitrification is greatly reduced at pH values below 4.2-4.4, although nitrification has been observed at low soil pH due to the presence of pH-neutral micro-sites (De Boer *et al.*, 1992; De Boer and Kowalchuk, 2001; Troelstra *et al.*, 1990). Negative effects of sod cutting on nitrification may therefore be primarily responsible for the NH_4^+ accumulation in the soil following sod cutting. This implies that additional restoration measures after sod cutting should be aimed at increasing nitrification. The application of lime to increase soil pH and subsequently nitrification rates (Roelofs *et al.*, 1996) might be a successful measure.
The aim of our research was to determine if sod cutting affects nitrification in soils from dry and wet heathlands. Furthermore, we experimentally tested the hypothesis that the application of lime following sod cutting would prevent or decrease the NH_4^+ accumulation by increasing nitrification.

MATERIALS AND METHODS

Study areas

The heathland area Havelte-Oost (HO) is located 8 km north of Meppel (52°48'N, 6°13'E). The topsoil consists of sandy deposits with a low loam content interspersed with areas with shallow loam layers and both dry and wet heathlands and matgrass swards are present. Species-rich plant communities that occur in drier areas are of the Nardo-Galion saxatilis and Calluno-Genistion pilosae type and in wetter areas the Ericetum tetralicis subass. orchietosum and Gentiano pneumonanthes-Nardetum associations are present.
The nature reserve Leemputten (LP) is situated on the Veluwe in the centre of the Netherlands, about 8 km east of Ermelo and 65 km to the south of HO (52°16'N, 5°44'E). Soils consist of sandy deposits and loam layers from the Riss glacial period, but they are highly variable and consequently, so is the species composition of the vegetation. Species-rich communities like Ericetum tetralicis subass. orchietosum,

Gentiano pneumonanthes-Nardetum and Cirsio dissecti-Molinietum, are intermingled with species-poor vegetation, in which *Calluna vulgaris*, *Erica tetralix* and *Molinia caerulea* are dominant.
Many of the target species for restoration, e.g. *Cirsium dissectum*, *Dactylorhiza maculata*, *Drosera intermedia*, *Drosera rotundifolia*, *Epipactis palustris*, *Gentiana pneumonanthe*, *Lycopodiella inundata*, *Narthecium ossifragum*, *Parnassia palustris*, *Pedicularis sylvatica*, *Rhynchospora alba*, *Rhynchospora fusca* and *Succisa pratensis* have now disappeared from large parts of both study areas. Nomenclature follows Schaminée *et al.* (1995) and Schwertz *et al.* (1996) for plant communities and Van der Meijden (1996) for vascular plants.

Sod cutting and lime treatments

In 2000, nine plots of 2 x 2 m were laid out within a part of the dry heathland of HO that had been treated by sod cutting in the autumn of 1999. This part had been dominated by *Molinia* prior to sod cutting. To four of these plots 200 g m^{-2} of Dolokal (80% $CaCO_3$, 20% $MgCO_3$) was applied. Within the wet heath of HO, two zones could be distinguished: one zone was dominated by *Molinia*, the other by *Erica.* In both zones four plots of 2 x 3 m were sod-cut in 2000. In autumn 2001 sod cutting was repeated in eight new 1 m^2 plots in the *Molinia*-dominated zone of HO, of which four plots were subsequently treated with 200 g m^{-2} ground limestone (100% $CaCO_3$). In LP sod cutting was carried out in four *Molinia*-dominated plots of 2 x 3 m in spring 2000. Liming was not carried out in this area. Instead, an area was marked in a species-rich Gentiano pneumonanthes-Nardetum vegetation. This area served as a non-acidified reference situation (soil pH was around 5.5). In all sites, four or five plots were established in the not sod-cut (control) vegetation. In the dry heath of HO, these control plots were situated in an area which was not completely dominated by *Molinia*, but where *Calluna* was also still abundant.

Effects of sod cutting and liming on potential net nitrification

Potential net nitrification was measured in soil incubation experiments lasting 6 weeks, in which soil suspensions were supplied with additional NH_4^+ (De Boer *et al.*, 1992). One sample of the upper 5 cm of soil was collected from each of the treatments: control, sod-cut and sod-cut + limed. Soil sampling was carried out in 2001, 2002 and 2003 in LP, the wet and dry heath of HO, respectively. Replicate samples per treatment were mixed and homogenised. In LP, soil samples of deeper soil layers (5-10 cm) of the acidified and of the non-acidified reference site were collected as well. An equivalent of 2.5 g dry soil of each mixed sample was weighed in erlenmeyer flasks (four or five replicates per treatment). The soil samples were incubated with 100 ml of a mineral solution, containing KH_2PO_4, $CaCl_2$, $MgSO_4.7H_2O$ (0.2 mM each) and 2.5 mM of $(NH_4)_2SO_4$. All flasks were placed on a light-covered rotary shaker (RO 20, Gerhardt, Bonn, Germany) in a climate chamber at 50 rpm. Temperature and humidity of the climate chamber were, on average, 20°C and 70%, respectively. The pH of the soil solutions was adjusted to pH 6 using Na_2CO_3, weekly. Prior to these adjustments 5 ml of the soil solutions were collected, filtered through a Whatman GF/C filter and stored at -20°C until further analysis. NO_3^- and

NH_4^+ concentrations of the soil solutions were measured on a continuous flow analyser (Skalar 40, Skalar Analytical BV, Breda, The Netherlands).
The soil moisture content of all soil samples was measured prior to the incubation experiments by drying 15 g of fresh soil at 105°C for 24 hours. Initial pH values of the incubated soil solutions were determined using a SenTix 41 pH-electrode.

Effects of sod cutting and liming on soil chemistry

Soil samples of the upper 5 cm of the control, sod-cut and sod-cut + limed plots of the dry and wet heath of HO (sod-cut and limed in 2001) were regularly collected during a one-year period following liming. In the wet heath, three samples were collected from each plot, which were transported to the laboratory in a cool box, stored at 4°C and processed within two days. After mixing and homogenising the three samples from each plot, 15 g of fresh soil was extracted on a rotary shaker (100 rpm) for 1 h with 100 ml demineralized water or 0.2 M KCl. The pH of the soil solution was measured and the samples were subsequently centrifuged for 5 min at 4000 rpm. The supernatants were filtered and stored as above. Soil moisture contents were measured after drying 15 g of fresh soil at 105°C for 24 hours. Ca^{2+}, Mg^{2+}, Al^{3+}, K^+, NH_4^+, NO_3^-, PO_4^{3-} and SO_4^{2-} concentrations were analysed colorimetrically using the Skalar continuous flow analyser.
In the dry heath, one soil sample was collected from each plot. The replicate soil samples of the different treatments were mixed, homogenised and analysed as described above. To analyse salt extractable ion concentrations, 0.2 M NaCl was used. Nutrient concentrations were measured on an inductive-coupled plasma emission spectrophotometer (ICP, Spectroflame Flame VML2).

Statistics

Statistical tests were carried out using SPSS 8.0 (SPSS Inc. 1989-1997). Data on potential net nitrification were compared with one-way ANOVA tests. If necessary, data were logarithmically transformed to stabilize variances between groups. *Post-hoc* Tukey tests were carried out to test for significant differences between treatments. Multivariate linear regression with the variables potential nitrification, soil pH, soil moisture and study area was also carried out. To test for significant correlations between potential nitrification and the other variables, Pearson correlation coefficients were calculated. Data on soil NH_4^+ and NO_3^- concentrations of the wet heath of HO were tested by means of repeated measures (General Linear Models). Because significant interactions existed between treatments and time, one-way ANOVA tests were carried out for each separate sample date. Data on NH_4^+ and NO_3^- concentrations of the dry heath of HO, as well as the other soil chemistry variables of both areas, were averaged over the one-year study period and tested for significant differences with one-way ANOVA tests. In all cases *Post-hoc* Tukey tests were carried out to test for significant differences between treatments.

RESULTS

Effects of sod cutting on nitrification

The potential net nitrification of soil from the control plots differed between the sites within both study areas (**table 1a**). Net nitrification was low in the *Calluna/Molinia*-dominated dry heath and in the *Erica*-dominated zone of the wet heath of HO (1.2 and 0.2 µmol NO_3^- g^{-1} dry soil after 6 weeks, respectively). The control plots of both wet heath sites dominated by *Molinia* had significantly greater net NO_3^- production than the two former sites (F = 60.26, p < 0.001). Sod cutting of the heath vegetation did not result in decreased potential net nitrification in the *Calluna/Molinia*-dominated dry heath, nor in the *Erica*-dominated wet heath at HO (**table 1a**). However, a significant lower potential net NO_3^- production after sod cutting was found in both *Molinia*-dominated wet heaths. In HO, potential net nitrification decreased almost twenty-fold and the decrease was even more substantial in LP.

Table 1: Cumulative NO_3^- production (+ s.e.) in µmol g^{-1} dry soil of control and sod-cut soils **(a)** and in sod cut and sod-cut +limed soils **(b)** after 6 weeks of incubation. The number of replicates (n), T-test t and p values are also indicated, with significant differences in bold. HO = Havelte-Oost, LP = Leemputten. *M* = *Molinia*-dominated, *E* = *Erica*-dominated, *C/M* = dominated by *Calluna* and *Molinia* (control), or *Molinia* (sod-cut). In the wet heath of HO, samples were collected from the plots sod-cut in 2000 and 2001.

	Control		Sod-cut		Sod-cut +limed				
	Mean	s.e.	Mean	s.e.	Mean	s.e.	n	T-test t	p
(a)									
HO-Dry heath *C/M*	1.2	0.5	5.4	2.3			5	-1.791	0.142
HO-Wet heath *E*	0.2	0.1	0.4	0.3			5	-0.652	0.533
HO-Wet heath *M* 2000	28.1	6.8	1.6	1.2			5	3.859	**0.005**
LP-Wet heath *M*	15.2	2.5	0.4	0.3			4	5.888	**0.001**
(b)									
HO-Dry heath *M*			5.4	2.3	48.7	1.7	5	-15.284	**<0.001**
HO-Wet heath *M* 2001			12.2	4.4	25.8	4.4	4	-2.191	0.071

Effects of liming of sod-cut plots on nitrification

Liming of the sod-cut plots increased the potential net nitrification in the dry and wet heaths of HO compared to sod cutting alone (**table 1b**). This increase was significant in the dry heath (p < 0.001) and substantial, but not significant, in the wet heath (p = 0.071). In LP potential net nitrification in the upper layer of the weakly buffered Gentiano pneumonanthes-Nardetum site was significantly greater than of the acidified site which was dominated by *Molinia* (p < 0.001; 61 and 15 µmol NO_3^- g^{-1} dry soil after 6 weeks, respectively; **figure 1**). This significant difference between the sites was also found in the 6-10 cm soil layer (p < 0.001). Potential net nitrification

also significantly decreased with increasing soil depth at both LP sites (with about 45% in both sites). Sod cutting of the *Molinia*-dominated site further reduced potential net nitrification to values as low as 0.4 µmol NO_3^- g^{-1} dry soil after 6 weeks, which was only 7% of that measured at the corresponding depth in the control *Molinia*-dominated wet heath site (6-10 cm).
When the data for all sites in both areas were pooled, it became clear that sod cutting did not significantly increase mean soil pH nor potential net nitrification compared to control soils (**figure 2**). However, soil moisture content was significantly lower after sod cutting (with or without lime).
The addition of lime to the sod-cut plots significantly increased mean NO_3^- production and soil pH compared to the other treatments. Stepwise multivariate linear regression showed that differences in potential net nitrification could to a large degree be explained by soil pH (coefficient = 24.5, $F = 75.7$, $p < 0.001$, $R^2 = 0.554$, n = 63). Soil pH and soil moisture combined explained 78% of the variation (coefficients = 30.8 and 1.0, respectively, $F = 103.0$, $p < 0.001$, $R^2 = 0.775$).

Effects of liming of sod-cut plots on soil chemistry

Liming of the sod-cut plots prevented the NH_4^+ accumulation in the topsoil of both the dry and wet heaths of HO (**figure 3a**). NH_4^+ concentrations of the sod-cut + limed plots in the wet heath of HO did not exceed 400 µmol kg^{-1} dry soil nor the proposed toxic threshold of c. 500 µmol kg^{-1} dry soil (Dorland *et al.*, 2003). However, during summer of 2002 toxic NH_4^+ concentrations were found in the sod-cut plots at HO (viz. 980 µmol kg^{-1} dry soil).

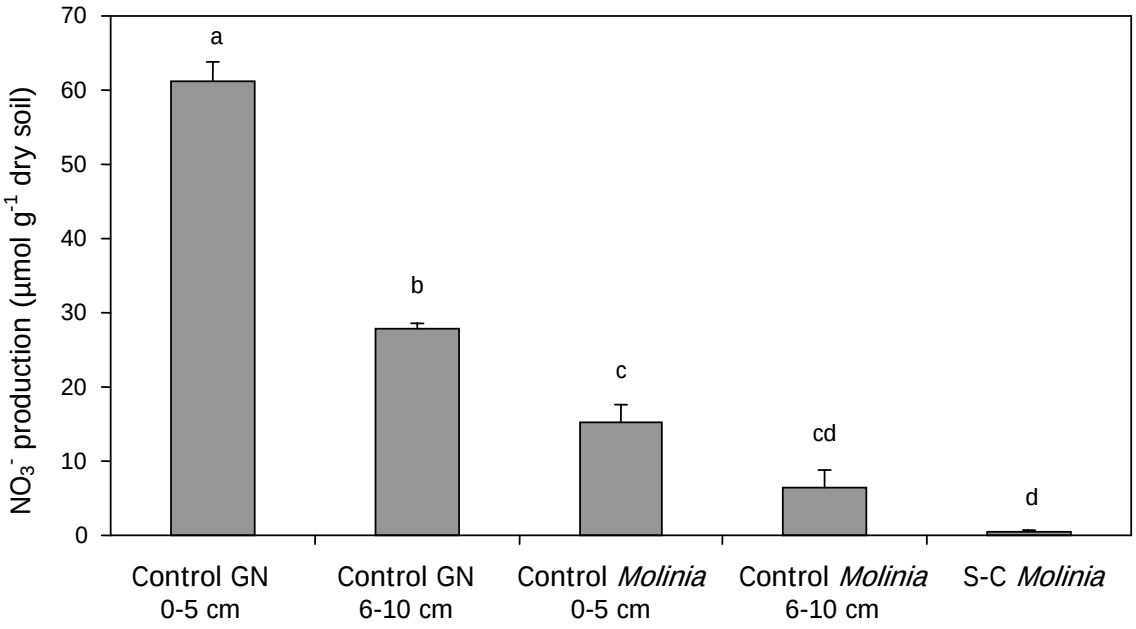

Figure 1: Potential net nitrification (+ s.e.) after 6 weeks of incubation (µmol g^{-1} dry soil) of weakly buffered soils of a Gentiano pneumonanthes-Nardetum (GN) vegetation and of acidified soils dominated by *Molinia* at two depths (0-5 cm and 6-10 cm) in LP. *Control* = soil of untreated vegetation; *S-C* = sod-cut soil (means + s.e.). Significant differences between treatments are indicated with different letters.

Soil NH_4^+ concentrations of the sod-cut + limed plots were significantly lower during this period and did not differ significantly from the control soils. In the dry heath of HO, NH_4^+ concentrations of the sod-cut plots were on average 906 µmol kg^{-1} dry soil, which was above the toxic threshold and significantly higher than those of the sod-cut + limed plots and of the control plots ($p < 0.001$; **table 2**).
Liming of the sod-cut plots of HO prevented the NH_4^+ accumulation presumably by increasing the nitrification in the topsoil layer. In the wet heath of HO significantly greater NO_3^- concentrations were found from April to July 2002 in the limed plots compared to those of the sod-cut plots (**figure 3b**). The NO_3^- concentrations in the soils of the control vegetation were negligible during the study period. The average yearly NO_3^- concentration of the sod-cut + limed plots in the dry heath of HO was also significantly higher than that of the sod-cut and control plots (812, 135 and 274 µmol kg^{-1} dry soil, respectively; **table 2**).
Besides reducing the accumulation of NH_4^+, the addition of lime after sod cutting had also beneficial effects on other soil factors. In both areas of HO the greatest soil pH values were found in the sod-cut + limed plots, although in the dry heath of HO this difference was not significant (**table 3**). Significant increases after liming were also found in soil Ca^{2+} concentrations compared to the control and sod-cut soils. In addition, the Al^{3+} concentrations and the Al^{3+}/Ca^{2+} and NH_4^+/NO_3^- ratios significantly decreased after liming of the sod-cut plots compared to sod cutting alone. Both ratios were even significantly lower compared to the control soils. Liming had no significant effect on mean Mg^{2+}, K^+, SO_4^{2-} and PO_4^{3-} concentrations or on the total mineral N concentration of the soil compared to the sod-cut plots.

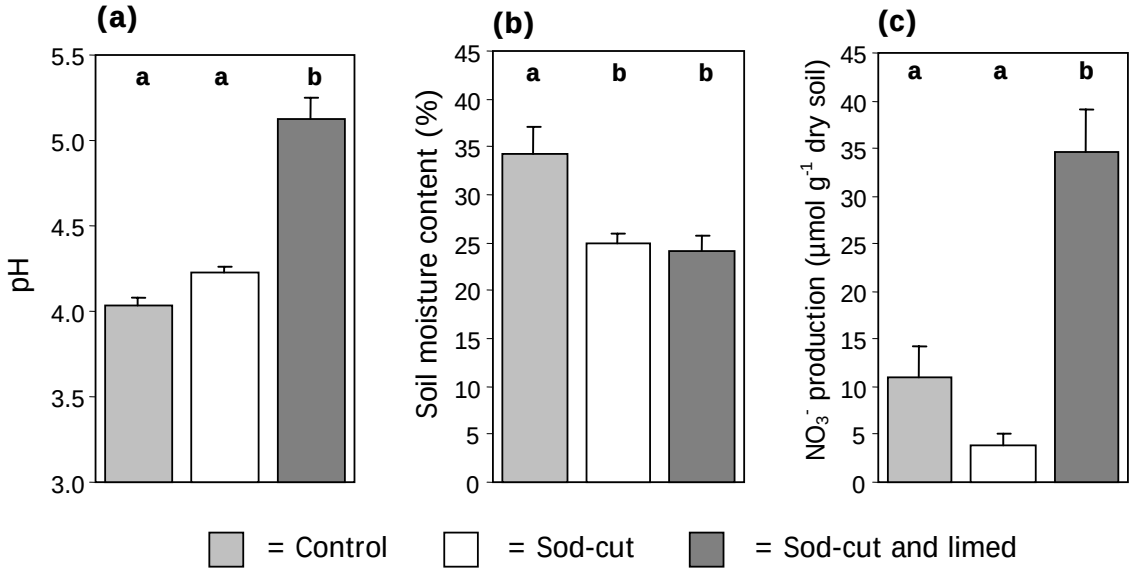

Figure 2: Initial soil pH **(a)**, soil moisture content **(b),** and potential net nitrification **(c)** after 6 weeks of incubation of control, sod cut, and sod-cut + limed heaths (+ s.e; n = 19, 23 and 21, respectively). Data of all sites of HO and LP were pooled. Significant differences between treatments are indicated with different letters.

Table 2: Mean values (+ s.e.) of 0.2 M NaCl-extractable NH_4^+ and NO_3^- concentrations of control, sod-cut and sod-cut + limed plots during 1 year after the application of lime (200 g m^{-2}) in the dry heath of HO (concentrations in µmol kg^{-1} dry soil). Different letters indicate significant differences between treatments. *P*-values are of one-way ANOVA tests, *n* = number of sampling events.

		NH_4^+			NO_3^-		
	n	Mean	s.e.	Sign.	Mean	s.e.	Sign.
Control	4	474	170	a	274	69	a
Sod-cut	12	906	70	b	135	31	a
Sod-cut + limed	10	231	50	a	812	142	b
				$p < 0.001$			$p < 0.001$

DISCUSSION

Sod cutting is an effective restoration measure for removing excess nutrients from an eutrophicated system and has therefore often been applied to restore various types of ecosystems such as heathlands (Britton *et al.*, 2000; Chytrý *et al.*, 2001; De Graaf *et al.*, 1998b), fens (Beltman *et al.*, 2001; Bootsma *et al.*, 2002) and grasslands (Jansen and Roelofs, 1996; Oomes and Van der Werf, 1996). However, recent research has shown that shortly after sod cutting of dry and wet heathlands, soil NH_4^+ concentrations increased dramatically (De Graaf *et al.*, 1998a; Dorland *et al.*, 2003). In this study, NH_4^+ accumulation was also found in the dry and wet heath plots at HO (**table 2; figure 3**), with maximum concentrations reached approximately 10 months after sod cutting and ranged from ca. 600-1000 to even 1000-1200 µmol kg^{-1} dry soil in the wet and dry heaths, respectively. High NH_4^+ concentrations in the soil of dry and wet heaths and matgrass swards will hamper germination and growth of many endangered plant species, for example *Cirsium dissectum* and *Succisa pratensis* (Dorland *et al.*, 2003; Lucassen *et al.*, 2003) and *Arnica montana* (De Graaf *et al.*, 1998a). As a consequence, the success of restoration of these former hot spots of diversity in the heath landscape will be less following traditional sod cutting methods. The toxic effects of high NH_4^+ concentrations will be even more pronounced in soils of low pH (Lucassen *et al.*, 2003; Van den Berg, personal observation), as is currently the case in most acidified Dutch heathlands.

We expected that NH_4^+ would accumulate in sod-cut heath soils, because sod cutting might negatively affect the nitrifying activity. Our results showed that this seems to be the case in the *Molinia*-dominated wet heath areas in both HO and LP. Potential net nitrification was low in heathlands in which dwarf-shrubs were (co)dominant and did not decrease further following sod cutting. The differences in nitrifying activity between heathlands that are dominated by grasses or by dwarf-shrubs have also been found in other studies (De Boer *et al.*, 1989; Troelstra *et al.*, 1990). De Boer *et al.* (1989) attributed these differences to the properties of the nitrifying micro-organisms present. In soils with low NO_3^- production, acid-sensitive, ammonium oxidizing bacteria were dominant, whereas acid-tolerant ammonium oxidizing bacteria were dominating in soils with rapid nitrate production. In our study areas, the latter type might have been dominating in the soils of the *Molinia*-dominated

heaths and the former in the *Erica*- or *Calluna*-dominated heaths. Acid-tolerant nitrification is of particular importance in heathland soils (De Boer and Kowalchuk, 2001).

Differences in the chemical composition of the soil organic material between dwarf-shrub dominated and degraded grass-dominated heathlands will further contribute to differences in nitrifying activity. C:N ratios of *Erica*- or *Calluna*-dominated areas are generally greater than those of grass-dominated heaths (Aerts and Berendse, 1988; Berendse, 1990), as was also found for both of our study areas (Dorland *et al.*, 2003). Since decomposition decreases with increasing C:N ratios (Berendse, 1990; Van Vuuren *et al.*, 1992), less NH_4^+ will be available in the dwarf-shrub dominated areas, hence decreasing nitrification. Such a negative relationship between nitrification and soil C:N ratio was also found by Gundersen *et al.* (1998) and Bengtsson *et al.* (2003). Here, in 2000, mean NH_4^+ concentrations following sod cutting were 350 μmol kg^{-1} dry soil in the *Molina*-dominated wet heath of HO, but only 70 μmol kg^{-1} dry soil in the *Erica*-dominated part (Dorland *et al.*, 2003). Since sod cutting is generally applied in degraded grass-dominated heathlands (viz. areas with low C:N ratios) and not in areas that are still dominated by dwarf shrubs, the negative effects of sod cutting on nitrification will play a major role in the accumulation of NH_4^+ in the soil.

We tested the prediction that the application of lime after sod cutting would result in decreased NH_4^+ accumulation. Liming successfully prevented NH_4^+ accumulation in both dry and wet heath plots, as maximum NH_4^+ concentrations did not reach toxic values. However, in the wet heath of HO a temporary increase in NH_4^+ concentrations was found in the sod-cut + limed plots compared to those of the sod-cut plots, although these differences were not significant (**figure 3**). This effect can be explained by increased decomposition as a result of significantly higher pH values after liming (e.g., Marschner and Wilczynski, 1991; Ulrich, 1983). Liming increases nitrogen mineralization when the C:N ratio of the organic matter is < 30, whereas immobilization will occur when this ratio is above 30 (Persson *et al.*, 1990). This can explain the temporary increase in NH_4^+ concentrations, because the C:N ratios of both study areas were below 30 (Dorland *et al.*, 2003). Apparently, decomposing micro-organisms were directly favoured by liming, whereas the effects of liming on nitrification were not yet noticeable. Similar results were reported by Neale *et al.* (1997), who found that the start of rapid nitrification lagged behind the increase in soil-pH by liming and the enhancement of mineralization. After this lag-period, nitrification was strongly stimulated, as can be concluded from the significantly higher NO_3^- concentrations in both the dry and wet heath of HO.

Increased potential net nitrification after liming of the sod-cut plots was also found in the incubation experiments. Liming increased the amount of NO_3^- produced in both the dry and wet heaths of HO (**table 1b; figures 2 and 3**). These results were in concordance with the differences in potential net nitrification between the untreated, weakly-buffered and the acidified sites in LP. Potential net nitrification was significantly greater in the former site which had a soil pH of 5.6, compared to that of the acidified, *Molinia*-dominated, site with a soil pH of 3.8 (**figure 1**). These differences were found for the topsoil layers, as well as in the 6-10 cm soil layers.

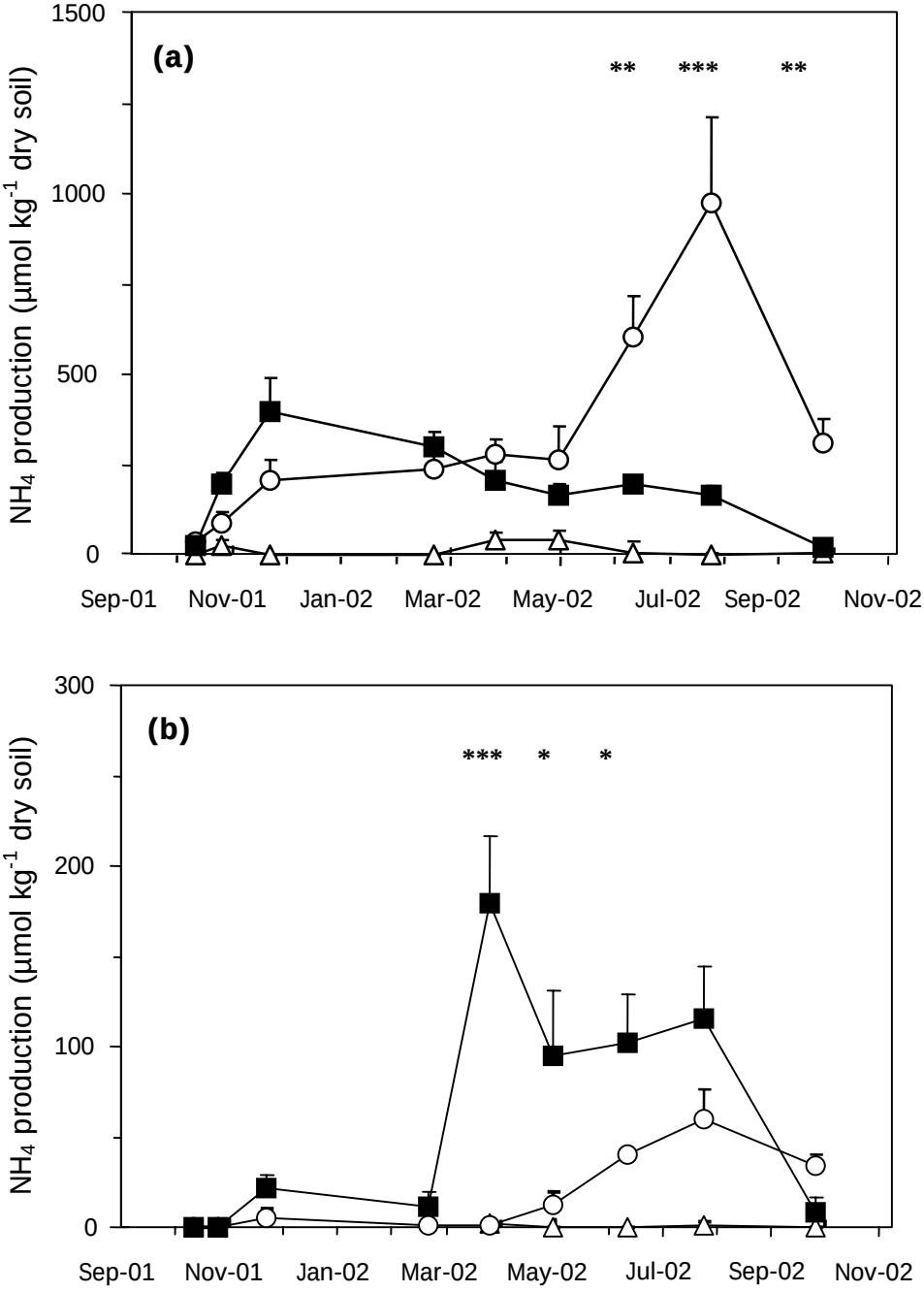

Figure 3: Soil 0.2 M KCl-extractable NH_4 concentrations **(a)** and demineralized water exchangeable NO_3 concentrations **(b)** of the wet heath HO (means + s.e.). Open circles = sod-cut, filled squares = sod-cut + limed; open triangles = control soil. Significant differences between sod-cut and sod-cut + limed treatments are indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 3: Mean values for soil chemistry (+ s.e.) of control, sod-cut and sod-cut + limed plots over a 1 year period after the application of lime (200 g m^{-2}) in the dry and wet heath of HO. K^+, Al^{3+} and PO_4^{3-} are demineralized water-exchangeable concentrations; Ca^{2+}, Mg^{2+}, NH_4^+ and NO_3^- are 0.2 M NaCl or KCl-extractable concentrations for the dry and wet heath, respectively (all in µmol kg^{-1} dry soil). Different letters indicate significant differences between treatments, *p*-values are of one-way ANOVA tests, *n* = number of sampling events.

	n	pH	Ca^{2+}	Mg^{2+}	K^+	Al^{3+}	Al^{3+}/Ca^{2+}	PO_4^{3-}	NH_4^+/N	N_{tot}
Dry heath										
Control	4	3.97 ± 0.08^a	653 ± 59^a	334 ± 39^a	224 ± 44^b	107 ± 8^{ab}	0.17 ± 0.03^b	5.0 ± 1.1^a	1.7 ± 0.4^b	747 ± 227^a
Sod-cut	12	4.50 ± 0.09^b	820 ± 97^a	258 ± 19^a	47 ± 5^a	123 ± 9^b	0.15 ± 0.02^b	9.7 ± 2.5^a	10.0 ± 1.8^c	1040 ± 74^a
Sod-cut + Limed	10	4.74 ± 0.15^b	6237 ± 850^b	1060 ± 142^b	72 ± 17^a	85 ± 10^a	0.02 ± 0.01^a	4.2 ± 0.9^a	0.6 ± 0.2^a	1044 ± 117^a
		$p = 0.007$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.022$	$p < 0.001$	$p = 0.051$	$p < 0.001$	$p = 0.291$
Wet heath										
Control	2	4.34 ± 0.02^a	1947 ± 447^a	869 ± 134^a	181 ± 48^a	124 ± 2^a	0.20 ± 0.01^b	0.7 ± 0.1^a	6.8[1]	19 ± 5^a
Sod-cut	4	4.46 ± 0.01^a	1642 ± 368^a	712 ± 158^a	219 ± 11^a	229 ± 19^b	0.17 ± 0.04^b	2.2 ± 0.3^b	$82.2 \pm 27\ 2^b$	340 ± 61^b
Sod-cut + Limed	4	5.25 ± 0.08^b	11723 ± 1034^b	789 ± 110^a	205 ± 42^a	158 ± 3^a	0.03 ± 0.01^a	1.1 ± 0.3^{ab}	3.2 ± 0.5^a	244 ± 26^b
		$p < 0.001$	$p < 0.001$	$p = 0.786$	$p = 0.693$	$p = 0.003$	$p = 0.002$	$p = 0.020$	$p = 0.002$	$p < 0.001$

[1] Value of one sampling event only, so no statistical tests were carried out.

Multiple regression analyses indicated that soil pH was positively correlated with NO_3^- production and the most important soil variable in predicting nitrification rates. Whether this was a causal effect remains unclear, because higher soil pH following liming might have increased nitrification indirectly by enhancing mineralization and, consequently, NH_4^+ availability. Troelstra *et al.* (1990) found no significant correlation between NO_3^- production and soil pH for 17 Dutch heathland areas, but a highly significant correlation for NO_3^- production and mineralization. Most important is that liming of sod-cut plots thus prevented the accumulation of NH_4^+ in the soil of dry and wet heaths. Toxic NH_4^+ concentrations for the target heath species were not reached and their germination and establishment would therefore not be hampered.

The question whether the observed decrease in potential net nitrification after sod cutting resulted from the removal of the nitrifying bacteria in the topsoil layer, or from the creation of unsuitable soil conditions for nitrification after sod cutting, may now be answered in more detail. Potential net nitrification decreased with increasing soil depth (**figure 1**), which is in accordance with the presumption that nitrifying bacteria are present mainly in topsoil layers (FH layer; Troelstra *et al.*, 1990). This suggests that sod cutting might remove most of the nitrifying bacteria. However, our results showed that liming increased NO_3^- production successfully in sod-cut soils of *Molinia*-dominated heathlands. The nitrification rates of sod-cut + limed soils were comparable to those of the 6-10 cm soil layer of the non-acidified of LP (viz. 15-30 μmol g^{-1} dry soil; **table 1b; figure 1**). Direct removal of nitrifying bacteria by sod cutting cannot be the only explanation for decreased nitrification following sod cutting. Reduced NO_3^- production, as a result of less suitable soil conditions for nitrification after sod cutting, will also be important. For example, the decrease in soil moisture content of sod-cut soils (**figure 2**), which was also found in **chapter 6** of this theis, might reduce nitrification in heathlands.

The soil moisture content was positively correlated with nitrification and explained in our multivariate regression model, together with soil pH, 78% of the variation in potential net nitrification. Positive correlations between soil moisture content and nitrification were also found for forest soils (Davidson *et al.*, 1993; Krave *et al.*, 2002; Owen *et al.*, 2003). Soil moisture contents of sod-cut + limed soils were also significantly lower compared to control soils (**figure 2**), but the negative effects of decreased soil moisture on nitrification were overcome by the higher soil pH, which is shown to have the most effect on nitrification. Other soil variables that are influenced by sod cutting are the organic matter content, humic acid concentrations (**chapter 6**), soil temperature and light penetration. How these changes might affect nitrification in heath soils remains unknown.

Liming prevented the NH_4^+-accumulation in the soil by increasing nitrification, moreover, it had several other effects on soil chemistry, from which endangered plant species may benefit in the future. Significantly higher pH values and base cation concentrations (mainly Ca^{2+}) were found and Al^{3+}/Ca^{2+} and NH_4^+/NO_3^- ratios were significantly lower in the sod-cut + limed plots (**table 3**). It can be concluded that after liming of sod-cut plots, soil conditions would be successfully restored and appropriate conditions would exist for germination and establishment of the endangered heath species. The success of restoration of former species-rich dry heath

and wet heaths and matgrass swards, in which seed availability of the target species is not limiting, can thus be increased when sod cutting is combined with the application of lime. Where seed limitation is an issue, creating appropriate soil conditions for the endangered plant species is a prerequisite for successful heathland restoration and conservation.

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REFERENCES

Aerts, R., Berendse, F. 1988. The effect of increased nutrient availability on vegetation dynamics in wet heathlands. *Vegetatio*, 76: 63-69.

Aerts, R., Heil, G.W. 1993. *Heathland: Patterns and Processes in a Changing Enivronment*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Bakker, J.P. 1989. *Nature Management by Grazing and Cutting*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Bakker, J.P., Poschlod, P., Strykstra, R.J., Bekker, R.M., Thompson, K. 1996. Seed banks and seed dispersal: Important topics in restoration ecology. *Acta Botanica Neerlandica*, 45: 461-490.

Beltman, B., Van den Broek, T., Barendregt, A., Bootsma, M.C., Grootjans, A.P. 2001. Rehabilitation of acidified and eutrophied fens in the Netherlands: Effects of hydrologic manipulation and liming. *Ecological Engineering*, 17: 21-31.

Bengtsson, G., Bengtson, P., Månsson, K.F. 2003. Gross nitrogen mineralization-, immobilization and nitrification rates as a function of soil C/N ratio and microbial activity. *Soil Biology andBiochemistry*, l35: 143-154.

Berendse, F. 1990. Organic-matter accumulation and nitrogen mineralization during secondary succession in heathland ecosystems. *Journal of Ecology*, 78: 413-427.

Bobbink, R., Ashmore, M., Braun, S., Flückiger, W., Van den Wyngaert, I.J.J. 2003. Empirical nitrogen critical loads for natural and semi-natural ecosystems: 2002 update. In: *Empirical critical loads for nitrogen. Proceedings of the expert workshop 2002*. *Environmental Documentation No. 164*. SAEFL, Berne, Switzerland, pp. 43-170.

Bobbink, R., De Graaf, M.C.C., Verheggen, G.M., Roelofs, J.G.M. 1998a. Heeft het heischrale milieu in Nederland nog toekomst? In: Bobbink, R., Roelofs, J.G.M., Tomassen, H.B.M. (Eds.). *Effectgerichte maatregelen en behoud biodiversiteit in Nederland*, pp. 131-159.

Bobbink, R., Heil, G.W., Raessen, M.B.A.G. 1992. Atmospheric deposition and canopy exchange processes in heathland ecosystems. *Environmental Pollution*, 75: 29-37.

Bobbink, R., Hornung, M., Roelofs, J.G.M. 1998b. The effects of air borne nitrogen pollutants on species diversity in natural and seminatural European vegetation. *Journal of Ecology*, 86: 717-738.

Bootsma, M.C., Van den Broek, T., Barendregt, A., Beltman, B. 2002. Rehabilitation of acidified floating fens by addition of buffered surface water. *Restoration Ecology,* 10: 112-121.

Britton, A.J., Marrs, R.H., Carey, P.D., Pakeman, R.J. 2000. Comparison of techniques to increase *Calluna vulgaris* cover on heathland invaded by grasses in Breckland, South East England. *Biological Conservation,* 95: 227-232.

Chytrý, M., Sedláková, I., Tichý, L. 2001. Species richness and species turnover in a successional heathland. *Applied Vegetation Science,* 4: 89-96.

Davidson, E.A., Matson, P.A., Vitousek, P.M., Riley, R., Dunkin, K., Garciamendez, G., Maass, J.M. 1993. Processes regulating soil emissions of NO and N_2O in a seasonally dry tropical forest. *Ecology*, 74: 130-139.

De Boer, W., Klein Gunnewiek, P.J.A., Troelstra, S.R., Laanbroek, H.J. 1989. Two types of autotrophic nitrification in acid heathland humus. *Plant Soil*, 199: 229-235.

De Boer, W., Kowalchuk, G.A. 2001. Nitrification in acid soils: micro-organisms and mechanisms. *Soil Biology and Biochemistry*, 33: 853-866.

De Boer, W., Tietema, A., Klein Gunnewiek, P.J.A., Laanbroek, H.J. 1992. The chemolithotrophic ammonium-oxidizing community in a nitrogen-saturated acid forest soil in relation to pH-dependent nitrifying activity. *Soil Biology and Biochemistry,* 24: 229-234.

De Graaf, M.C.C., Bobbink, R., Roelofs, J.G.M., Verbeek, P.J.M. 1998a. Differential effects of ammonium and nitrate on 3 heathland species. *Plant Ecology*, 135: 185-196.

De Graaf, M.C.C., Verbeek, P.J.M., Bobbink, R., Roelofs, J.G.M. 1998b. Restoration of species-rich dry heaths. The importance of appropriate soil conditions. *Acta Botanica Neerlandica,* 47: 89-111.

De Graaf, M.C.C., Verbeek, P.J.M., Verheggen, G.M., Bobbink, R., Roelofs, J.G.M. 1995. Experimental liming of acidified heathlands and grasslands: effects on soil chemistry and vegetation. In: Jenkins, A., Ferrier, R.C., Kirby, C. (Eds.). *Ecosystem Manipulation Experiments: scientific approaches, experimental design and relevant results*. European Commission, Luxembourg, pp. 129-137.

Dorland, E., Bobbink, R., Messelink, J.H., Verhoeven, J.T.A. 2003. Soil ammonium accumulation after sod cutting hampers the restoration of degraded wet heathlands. *Journal of Applied Ecology,* 40: 804-814.

Erisman, J.W. 1992. *Atmospheric Deposition of Acidifying Compounds in the Netherlands*. Dissertation, Utrecht University, Utrecht, The Netherlands.

Gundersen, P., Callesen, I., De Vries, W. 1998. Nitrate leaching in forest ecosystems is related to forest floor C/N ratios. *Environmental Pollution*, 120: 403-407.

Houdijk, A.L.F.M., Verbeek, P.J.M., Van Dijk, H.F.G., Roelofs, J.G.M. 1993. Distribution and decline of endangered herbaceous heathland species in relation to the chemical composition of the soil. *Plant Soil*, 148: 137-143.

Jansen, A.J.M., De Graaf, M.C.C., Roelofs, J.G.M. 1996. The restoration of species-rich heathland communities in the Netherlands. *Vegetatio*, 126: 73-88.

Jansen A.J.M., Roelofs, J.G.M. 1996. Restoration of *Cirsio-Molinietum* wet meadows by sod cutting. *Ecological Engineering,* 7: 279-298.

Krave, A.S., van Straalen, N.M., van Verseveld, H.W. 2002. Potential nitrification and factors influencing nitrification in pine forest and agricultural soils in Central Java, Indonesia. *Pedobiologia*, 46: 573-594.

Lucassen, E.C.H.E.T., Bobbink, R., Smolders, A.J.P., Van der Ven, P.J.M., Lamers, L.P.M., Roelofs, J.G.M. 2003. Interactive effects of low pH and high ammonium levels responsible for the decline of *Cirsium dissectum* (L.) Hill. *Plant Ecology,* 165: 45-52.

Marschner, B., Wilczynski, A.W. 1991. The effect of liming on quantity and chemical

composition of soil organic matter in a pine forest in Berlin, Germany. *Plant Soil*, 137: 229-236.

Mitchell, R.J., Auld, M.H.D., Hughes, J.M., Marrs, R.H. 2000. Estimates of nutrient removal during heathland restoration on successional sites in Dorset, southern England. *Biological Conservation*, 95: 233-246.

Neale, S.P., Shah, Z., Adams, W.A. 1997. Changes in microbial biomass and nitrogen turnover in acidic organic soils following liming. *Soil Biology and Biochemistry*, 29: 1463-1474.

Oomes, M.J.M.,Van der Werf, A. 1996. Restoration of species diversity in grasslands: the effect of grassland management and changes in ground water level. *Acta Botanica Gallica,* 143: 451-461.

Owen, J.S., Wang, M.K., Wang, C.H., King, H.B., Sun, H.L. 2003. Net N mineralization and nitrification rates in a forested ecosystem in north-eastern Taiwan. *Forest Ecology and Management*, 176: 519-530.

Persson, T., Wiren, A., Andersson, S. 1990. Effects of liming on carbon and nitrogen mineralization in coniferous forest. *Water, Air and Soil Pollution,* 54: 351-364.

Roelofs, J.G.M., Bobbink, R., Brouwer, E., De Graaf, M.C.C. 1996. Restoration ecology of aquatic and terrestrial vegetation on non-calcareous sandy soils in the Netherlands. *Acta Botanica Neerlandica*, 45: 517-541.

Roem, W.J., Klees, H., Berendse, F. 2002. Effects of nutrient addition and acidification on plant species diversity and seed germination in heathland. *Journal of Applied Ecology*, 39: 937-948.

Schaminée, J.H.J., Van 't Veer, R., Van Wirdum, G. 1995. Oxycocco-Sphagnetea. In: Schaminée, J.H.J., Weeda, E.J., Westhoff, V. (Eds.). *De vegetatie van Nederland. Deel 2. Plantengemeenschappen van wateren, moerassen en natte heiden.* Opulus Press, Leiden, The Netherlands, pp. 287-304.

Schwertz, C.A., Schaminée, J.H.J., Dijk E. 1996 *Nardetea*. In: Schaminée, J.H.J., Weeda, E.J., Westhoff, V. (Eds.). *De Vegetatie van Nederland. Deel 3. Plantengemeendschappen van graslanden, zomen en droge heiden*. Opulus Press, Leiden, The Netherlands, pp. 263-286.

Snow, C.S.R., Marrs, R.H. 1997. Restoration of *Calluna* heathland on a bracken *Pteridium* infested site in North West England. *Biological Conservation*, 81: 35-42.

Troelstra, S.R., Wagenaar, R., Deboer, W. 1990. Nitrification in Dutch heathland soils. 1. General soil characteristics and nitrification in undisturbed soil cores. *Plant Soil*, 127: 179-192.

Ulrich, B. 1983. A concept of ecosystem stability and of acid depostition as driving force for destabilization. In: Ulrich, B., Pankrath, J. (Eds.). *Effects of accumulation of air pollutants in forest ecosystems*. Reidel Publishing Company, Dordrecht, The Netherlands, pp. 127-146.

Van Breemen, N., Burrough, P.A., Velthorst, E.J., Van Dobben, H.F., De Wit, T., Ridder, T.B. 1982. Soil acidification from atmospheric ammonium sulphate in forest canopy throughfall. *Nature*, 299: 548-550.

Van der Meijden, R. 1996. *Heukel's Flora van Nederland*. Wolters-Noordhoff B.V., Groningen, The Netherlands.

Van Vuuren, M.M.I., Aerts, R., Berendse, F., De Visser, W. 1992. Nitrogen mineralization in heathland ecosystems dominated by different plant species. *Biogeochemistry* 16: 151-166.

Chapter 6

HEATHLAND RESTORATION IN THE NETHERLANDS: EFFECTS OF TURF CUTTING DEPTH ON GERMINATION OF *ARNICA MONTANA*

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SUMMARY Germination experiments were conducted in a heathland after turf-cutting and in a climate chamber to investigate the effects of turf-cutting depth, aluminium toxicity and aluminium detoxification by humic acids and base cations on the germination and establishment of *Arnica montana*. Turfs were cut at three different depths, creating a gradient from organic to mineral soils. Germination and establishment of *A. montana* were negatively correlated with turf-cutting depth. The removal of organic matter resulted in a major decrease in organic fraction of the soil and its nutrients. It also resulted in a considerable decrease in moisture content and humic acids. Additional liming after turf-cutting increased germination and establishment in all plots and at all depths. Germination experiments under controlled conditions in a climate chamber revealed a significantly higher germination at low aluminium/calcium (Al:Ca) ratios. High Al:Ca ratios resulted in very poor germination, suggesting aluminium toxicity. Addition of humic acids increased germination, even at high Al/Ca ratios, suggesting immobilization of aluminium and humic acids. It is concluded that turf-cutting can have a marked effect on the success of heathland restoration. It results in the intended removal of the eutrophic layer, but also in the (unintentional) removal of much of the buffering mechanisms and/or aluminium-immobilizing compounds. Additional buffering and/or less deep turf-cutting can be necessary to allow germination and establishment of rare herbaceous species like *A. montana*.

INTRODUCTION

Species-rich heathlands (Calluno-genistion pilosae) and species-rich grasslands (Nardo-galion saxatilis) are being severely threatened in Western Europe and it is generally recognised that these ecosystems are in need of conservation and proper management. Many heathlands and species-rich grasslands in the Netherlands have seriously declined in terms of both area and biodiversity. In addition to factors like conversion to the purpose of forestry and agriculture, lack of proper management and fragmentation, one of the most serious threats in recent decades has been the atmospheric deposition of nitrogen and sulphur compounds (Bobbink *et al.*, 1992; Lee and Caporn, 1998). These compounds cause eutrophication and acidification of the soil, leading eventually to a transition from communities dominated by dwarf shrubs to those dominated by grasses (e.g. Heil and Diemont, 1983; Aerts and Heil, 1993; Bobbink *et al.*, 1998). Many characteristic herbaceous species like *Arnica montana*, *Thymus vulgaris* and *Antennaria dioica* have disappeared and their occurrence is at present restricted to only a few small populations (Fennema, 1992; Houdijk *et al.*, 1993).

Turf removal is a widely used management technique, as removal of the organic material helps to maintain an oligotrophic system (e.g. Werger *et al.*, 1983; Aerts and Heil, 1993; Snow and Marrs, 1997). Although turf-cutting has been demonstrated to be useful in restoring the acid-tolerant heathland vegetation, many herbaceous species have not reappeared after removal of the organic layer (De Graaf *et al.*, 1998). It has been suggested that germination was prevented by a decrease in soil pH and reduced base saturation, as a result of atmospheric deposition and subsequent acidification (Van Dam *et al.*, 1986; De Graaf *et al.*, 1998).

With ongoing acidification, heathlands and species-rich grasslands shift in buffer range from a cation exchange buffering (pH 4.5-6), towards an aluminium buffering (pH < 4.5; Scheffer and Schachtschabel, 1979). Consequently, aluminium availability strongly increases below pH 4.5 (Van Breemen *et al.*, 1982; Ulrich *et al.*, 1983, 1991; Ritchie, 1995). Previous research has revealed aluminium toxicity on plants (Foy *et al.*, 1978; De Graaf *et al.*, 1997). Fennema (1992) found higher concentrations of aluminium in the areas, where herbs like *A. montana* had disappeared. Others have found detoxification of aluminium by base cations (Korcak, 1990; Ryan *et al.*, 1994) and research by De Graaf *et al.* (1998) suggested that the aluminium/calcium ratio is one of the determinants of the success of restoration measures in Dutch heathlands.

Turf-cutting affects the physical a well as chemical parameters of the soil. Since the organic layers hold most of the soil moisture, turf-cutting will result in a reduction of the moisture content of the soil. Next to this, many organic compounds that are capable of complexing with (potential) toxic metals such as aluminium are present in the organic matter (e.g. Cronan *et al.*, 1978; Anderson, 1991; Beyer, 1996). Both soluble as well as insoluble organic compounds are important for the detoxification of aluminium (Ritchie, 1995 and references herein). Soluble organic matter includes humic substances that can form complexes with and detoxify aluminium. These include humic and fulvic acids (Currie *et al.*, 1996; Nasholm *et al.*, 1998). With the removal of the organic matter the amounts of aluminium complexing compounds like humic acids will be reduced. As a result, potentially toxic compounds and

elements that are detoxified by the organic complexes can have more pronounced effects after the organic material has been removed. Since natural detoxifying compounds in the soil are found in the organic layer, detoxification of aluminium could be expected to depend on turf-cutting depth, since more organic material is removed with deeper turf-cutting levels. Therefore, the success of restoration measures and the opportunities for germination and establishment of herbaceous species like *A. montana* could be expected to be related to turf-cutting depth and the amounts of detoxifying compounds present.
This paper presents the results of two germination experiments with the rare perennial herb *A. montana*. The first was a field experiment in which turf-cutting was applied at three different depths, representing the turf-cutting depths that are actually used in current restoration projects. Since turf-cutting at three different depths results in chemical as well as physical changes to the soil, a second germination experiment under controlled conditions was performed in a climate chamber, using a range of Al:Ca ratios and various amounts of humic acids. It was expected that aluminium addition would negatively affect the germination of *A. montana*. Furthermore, enhanced germination and establishment of *A. montana* were expected when aluminium was detoxified by calcium or humic acids, both in the laboratory experiments and in the field.

Materials and Methods

The species

A. montana is a perennial species characteristic of dry heathlands and dry, nutrient-poor, grasslands (Houdijk *et al.*, 1993; Ferguson, 1976). Seeds of *A. montana* were collected in a Dutch nature reserve (Havelterberg; 52°57'N, 6°15'E) in the autumn of 2001 and sterilised with sodium hypochlorite (1%, 10 minutes). Only dark and firm seeds were selected for further experiments, since these had proved most vital (data not shown).

Field experiments

Field experiments were carried out on a heathland dominated by grasses (chiefly *Molinia caerulea*), located 200 m from the *Arnica* population from which we had collected the seeds. At this site, turfs were cut manually at three different depths. Twelve plots of 50 x 50 cm were turf-cut to a depth of 15 cm, i.e., down to the mineral substrate. These plots formed the deepest turf-cutting condition and are referred to as 'mineral'. Twelve plots were turf-cut to a depth of 10 cm and referred to as 'intermediate'. In twelve plots only 5 cm of organic material was removed and these plots are referred to as 'organic'. All plots (turf-cutting depth) were selected at random. To reduce edge effects, the surrounding 15 cm of all plots were also cut to the corresponding depths. Six of the twelve plots for each depth were selected for chemical analysis of the soil, the remaining six being used for germination experiments. This was done to prevent disruption of the germination process by destructive soil sampling. Within each turf-cutting condition, six plots (three from

the germination part and three from the soil-analysis part) per depth were limed with $CaCO_3$ (200 gm^{-2}), the remaining six being used as control plots. Lime was added to plots which were selected at random within each turf-cut treatment. The quantity of 200 gm^{-2} $CaCO_3$ corresponds to liming treatments used in habitat restoration and conservation projects and has been found to have beneficial effects on soil chemistry and *A. montana* germination (De Graaf *et al.*, 1998).

Chemical analysis

Samples of the topsoil were taken with an auger (length 10 cm, diameter 2.5 cm) every four weeks for one year from 2001 to 2002. During the winter, soil samples were taken every 6-8 weeks. Samples were stored in polyethylene bags at 4°C until further analysis. Each soil sample was treated separately, resulting in three replicates per treatment. A portion of 35 g of fresh soil was mixed with 100 ml bi-distilled water or 100 ml 0.2 M NaCl solution in a 250 ml polyethylene bottle. After 1 hour of shaking at 100 rpm, pH was measured with a Radiometer type PHM 82 pH meter. The samples were then centrifuged for 20 minutes at 11,000 rpm and the supernatant was stored in polyethylene bottles at -28°C until further analysis. Water-extractable and exchangeable nutrient concentrations of Al, Ca, Mg and P were measured using an inductive-coupled plasma emission spectrophotometer (ICP; Jarrell Ash IL plasma-200). K concentrations were determined using flame photometry (Technicon Flame photometer IV). DOC fractions were defined as hydrophilic acids, humic acids and fulvic acids, as described by Impellitteri *et al.* (2002). The concentration of dissolved organic carbon (DOC) was measured in duplicate for all turf-cutting depths using a continuous-flow analyser (Skalar type 40). In addition, the amounts of humic acids were estimated from the light absorption values of the soil extracts at 450 nm (E_{450}) using a spectrophotometer (Shimadzu UV120-01). This estimation was based on the highly significant linear relation between light absorption by dissolved humic acidsand humic acid concentrations (measured at 450 nm; $R^2 = 0.992$, $d.f. = 35$, $p < 0.001$). Moisture content of the soil was measured after heating for 24 hours at 105°C. The organic fraction of the soil samples was determined by combustion for 4 hours at 550°C.

Germination experiment

In October 2001, 100 seeds per plot were sown and germination was assessed weekly. Seeds were recorded as 'germinating' as soon as breaking of the seed wall was observed. The seedlings were marked using small wooden sticks to be able to establish the total germination without making errors due to the death of seedlings and the subsequently germination of others during the period of measuring. 'Establishment' of the plants was assessed after one year as a percentage of the initial number of seeds sown.

Table 1: Mean values **(a)** and results of a general linear model procedure **(b)** on liming and turf-cutting depth on the germination, germination rate and establishment of seeds of *Arnica montana*. All variables followed a log-transformation prior to analysis.

(a) Mean values	Organic		Intermediate		Mineral	
	Lime	Control	Lime	Control	Lime	Control
Germination (%)	85	72	82	53	85	33
Germination rate (seeds day^{-1})	3.41	3.32	3.62	2.51	3.76	1.56
Establishment (%)	81	29	79	14	83	6

(b) Statistical results	Lime addition effect				Turf-cutting depth effect				Lime * depth interaction effect			
	d.f.	*MS*	*F*	*p-value*	*d.f.*	*MS*	*F*	*p-value*	*d.f.*	*MS*	*F*	*p-value*
Germination (%)	1	0.231	72.158	**0.000**	2	4.159E-02	12.996	**0.001**	2	4.106E-02	12.832	**0.001**
Germination rate (seeds day^{-1})	1	0.159	41.109	**0.000**	2	3.194E-02	8.267	**0.006**	2	5.012E-02	12.972	**0.001**
Establishment (%)	1	3.072	52.977	**0.000**	2	2.360E-01	4.070	**0.045**	2	2.520E-01	4.341	**0.038**

Laboratory experiments

Fifty *A. montana* seeds per petri dish were sown on Whatman glass microfibre filters (9 cm GF/D) soaked in different solutions. Each petri dish received a specific treatment and all treatments were replicated five times. Five different Al/Ca ratios (0, 0.5, 1, 5 and 15) and three levels of humic acids were used. A zero ratio treatment was used in which no Al or Ca was added. All other treatments had varying aluminium concentrations at constant calcium concentration of 100 µmol l^{-1}. All concentrations varied between 0 and 1500 µmol l^{-1}, representing very low to very high natural conditions. Ca was applied as either 0 or 100 µmol l^{-1}, both of which are low concentrations, representing the poorly buffered systems found in dry heathlands and acidic grasslands. Three levels of humic acids were applied to the Al/Ca ratios resulting in a total of 15 treatments. Humic acids were obtained from Jansen Chimica and added as 0.25 g l^{-1} and 0.0025 g l^{-1}. Control treatments contained 0 g l^{-1} humic acids. The solutions were adjusted for pH using HCl and pH was checked weekly. Aluminium was added as $AlCl_3$, while Ca was added in the form of $CaCl_2$. NaCl was used to balance ion concentrations in all treatments.
All petri dishes were placed in randomised order in a climate chamber with a temperature regime of 20-24°C during the light period (16 hours at a light intensity of 100 ± 10 µEm^{-2} s^{-1}, relative humidity 40-50%) and 15-19°C during the dark period (8 hours, relative humidity 85-90%). Germinated seeds (i.e., seeds with emerged radicle) were counted every two days to measure germination rate. Germination rate was expressed as the slope of the regression line through the germination curve from day 7 to 15 after the start of the germination experiment. After 8 weeks, when no further germination occurred, the total number of seedlings per petri dish was determined ('total germination').

Statistical analysis

Germination, germination rate and establishment were analysed using a two-way ANOVA performed with the General Linear Models (GLM) procedure after log-transformation, in order to fit a normal distribution. A repeated measures analysis was used to examine the response to additional liming and turf-cutting depth. All statistical analyses were performed with the SPSS 10.0 package (SPSS Inc., Chicago, USA).

RESULTS

Field experiments

The relationship between turf-cutting depth, lime addition and germination of *A. montana* is presented in **figure 1a**. A reduced germination with increasing turf-cutting depth was observed in the control plots. Total germination decreased from 72% in plots where 5 cm organic material had been removed to 53% and 33% in plots where 10 cm, or all (15 cm), of the organic material had been removed. The germination rate was consistently higher in the plots where some organic material

had been left and was highest in the 'organic' plots (**table 1**). Lime addition clearly improved germination and germination rate (**figure 1a; table 1**). The effect of lime was more pronounced in soils where more organic material had been removed ('intermediate' and 'mineral'), as is indicated by the lime * depth interaction effect in **table 1**.

The establishment of *A. montana* is presented as the percentage of seedlings present one year after sowing. *A. montana* seedlings established poorly in the plots without additional liming. Only 30% established in the 'organic' plots and lower establishment percentages were observed where more organic material had been removed, indicated by the less than 10% establishment in the mineral plots (**figure 1b**). Addition of lime, however, resulted in a high seedling establishment in all plots and all depths. Again, an interaction effect between lime and turf-cutting depth was found, illustrating that the beneficial effect of liming was greater where more organic material had been removed.

Soil parameters

Turf-cutting and liming both had strong effects on the soil parameters. Turf-cutting led to a considerable reduction of organic matter content (**figure 2; table 2**). Concentrations of dissolved organic compounds were significantly lower in the 'mineral' treatment (34.89 mg l^{-1}) than in the 'organic' treatment (55.56 mg l^{-1}; **table 3**), indicating a considerable reduction of humic substances, resulting from the removal of organic material. Light absorption was found to be negatively correlated with turf-cutting depth (**table 3**).

The light absorption of the soil extracts serves as an important indicator of the total amount of dissolved humic substances in the soil. These figures were consistent with the DOC measurements, indicating a decrease in dissolved humic substances as a result of the removal of organic material. Furthermore, reduced moisture content was observed after turf-cutting (**figure 3**).

The pH of the soil was significantly increased by liming (**figure 4; table 2**). Apart from this, turf-cutting down to the mineral layer also resulted in an increase in pH. This has also been found in other heathland soils (De Graaf *et al.*(1998) and can be attributed to the removal of the acid top soil.

Aluminium concentrations were found to be high in the 'organic' plots, compared with the concentrations in plots cut more deeply (**figure 5**). However, aluminium concentrations were measured using ICP, which measures all water-extractable aluminium, i.e., dissolved inorganic aluminium, as well as dissolved aluminium-organic complexes. Hence, these aluminium concentrations do not represent the Al^{3+} fraction in the soil. Lime had been added as $CaCO_3$ and had no effect on the aluminium concentration. It did, however, change the Al/Ca ratio significantly ($p < 0.05$, data not shown).

Laboratory experiments

No pH differences in the various solutions could be detected during the experiment: all solutions remaining at pH 3.5 (data not shown). The effects of different Al/Ca ratios and different humic acid levels on the germination of *A. montana* are shown in **figure 6**. Total germination and germination rate decreased significantly when the Al/Ca ratio was increased ($p < 0.001$). This effect was visible at all humic acids levels. Addition of humic acids improved germination and germination rate significantly at all Al/Ca ratios, except for the control treatment, where no positive effects could be detected. Germination was high in the petri dishes with low Al/Ca ratios or large

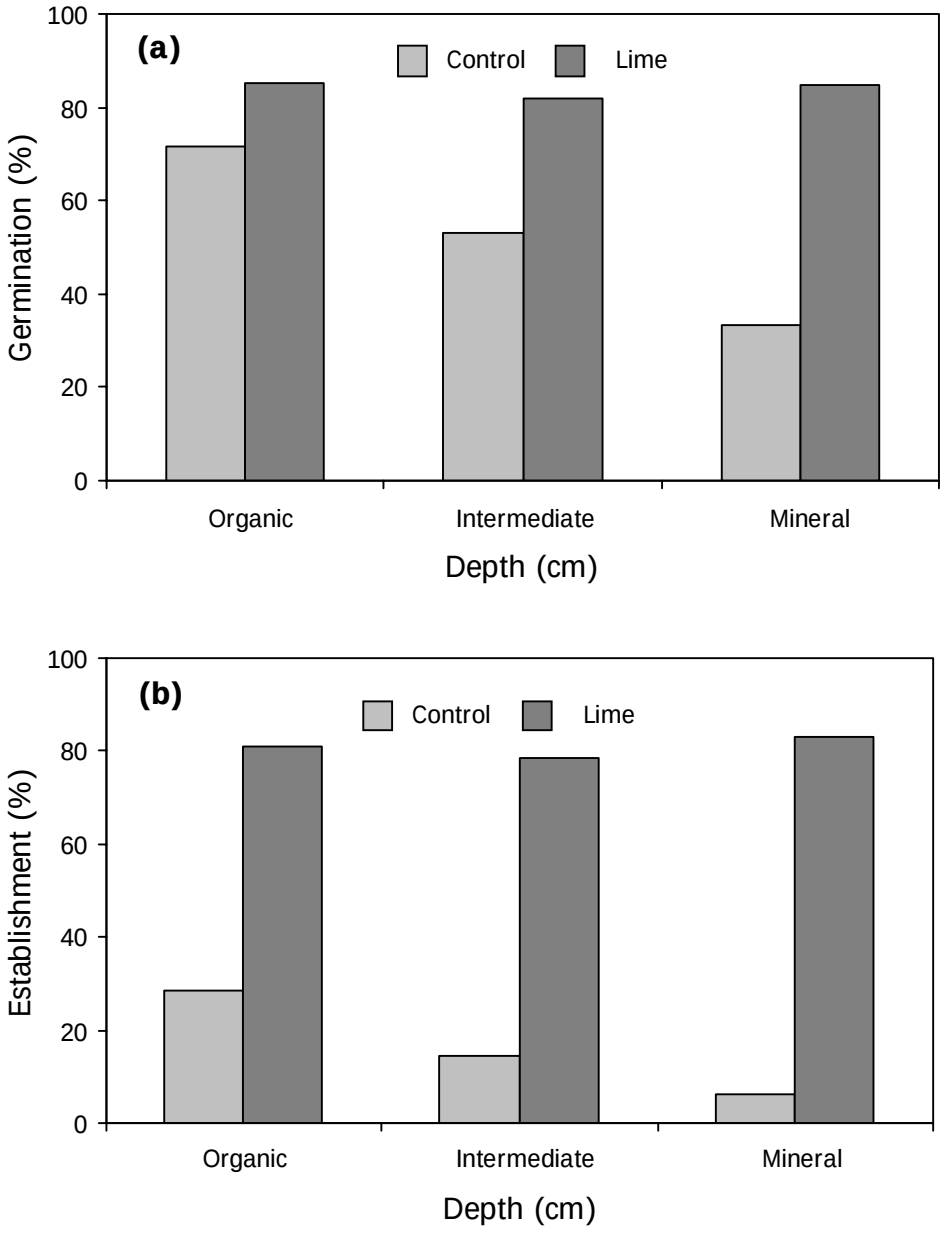

Figure 1: Mean germination percentage **(a)** and establishment percentage **(b)** of *Arnica montana* in a turf-cut heathland. Results after turf-cutting at three depths ('organic', intermediate' and 'mineral'), with and without additional liming are shown. Establishment was measured one year after sowing.

amounts of humic acids. At high Al/Ca ratios, the positive effects of humic acids on germination were more clearly indicated by the Al/Ca * humic acid interaction effect in **table 4**.
At the 0 g l^{-1} humic acid level, addition of aluminium and calcium had negative effects on germination, even when the ratio was only 0.5, whereas at higher humic acids levels (0.0025 and 0.25 g l^{-1}), addition of aluminium and calcium at a ratio of 0.5 had positive effects on germination.

Table 2: Repeated measures of analysis of variance (general linear models) of the effects of depth of turf-cutting and liming on different soil characteristics. F values are given, with their level of significance: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ^{ns} not significant; *d.f.* = 2, 1 and 11 for lime treatment, depth and time, respectively.

	Independent variable					
	Between subjects effects			*Within* subject effects		
	Lime	Depth	Lime * depth	Time	Time * lime	Time * depth
pH	130.938^{***}	24.229^{***}	10.509^{**}	6.377^{*}	0.956^{ns}	0.174^{ns}
Organic fraction (%)	0.009^{ns}	44.428^{***}	2.127^{ns}	0.410^{ns}	2.791^{ns}	0.447^{ns}
Soil humidity (%)	0.048^{ns}	8.576^{**}	0.812^{ns}	2.310^{ns}	0.628^{ns}	1.316^{ns}
Al $_{(H20)}$	2.520^{ns}	144.660^{***}	1.933^{ns}	0.002^{ns}	0.116^{ns}	0.417^{ns}
Al/Ca $_{(NaCl)}$	149.598^{***}	28.569^{***}	29.645^{***}	0.106^{ns}	0.190^{ns}	1.386^{ns}

Table 3: Mean values and results of a general linear model procedure on DOC and light absorption of soil extracts (measured at 450 nm) for three different turf-cutting depths. Different letters indicate significant differences at the $\alpha = 0.001$ level.

	DOC (mg l^{-1})	Light absorption (450 nm)
Organic	55.56^{a}	0.025^{a}
Intermediar	43.53^{ab}	0.019^{b}
Mineral	34.89^{b}	0.013^{c}

DISCUSSION

This paper reports on the chances of restoration and conservation of species-rich heathlands (Calluno-Genistion pilosae) and species-rich grasslands (Nardo-Galion saxatilis) after management measures like turf-cutting and additional liming. Topsoil removal resulted in a strong reduction of the amount of organic matter (**figure 2**) and nutrients (data not shown). This was consistent with the results from others (e.g. Berendse, 1990; Aerts and Heil, 1993; Diemont, 1994; Snow and Marrs, 1997) and it was concluded that turf-cutting successfully restored oligotrophic conditions.

However, germination and establishment of *A. montana* are negatively correlated with turf-cutting depth. A poor establishment of less than 10% was observed after one year in the plots, where all organic matter was removed. Similarly, De Graaf *et al.* (1998) found that turf-cutting successfully restored the acid-tolerant heathland vegetation, although many herbaceous species did not reappear after removal of the organic layer. Heathlands and species rich grasslands suffer from the atmospheric deposition of nitrogen- and sulphur-containing compounds and the subsequent acidification, resulting in low soil pH and very low base saturation. It was therefore suggested that turf-cutting as a sole management measure was inadequate and additional liming was needed to improve soil pH and base saturation (Van Dam *et al.*, 1986; De Graaf *et al.*, 1998). Therefore, additional liming was applied to the turf-cut heaths. Lime increased the base saturation (data not shown) and the pH of the soil significantly. The effect was most clear in the 'mineral' plots where pH increased from 3.5 to 5.5, shifting the soil from aluminium-buffering towards a cation exchange-buffering (Scheffer and Schachtschabel, 1979). Consequently, germination and establishment of *A. montana* in a turf-cut heathland were higher after lime addition. At all depths germination was high and comparable to germination under ideal conditions measured in a laboratory (data not shown). These results were consistent with those obtained previously by De Graaf *et al.* (1998), who found higher germination and establishment in turf-cut as well as limed heaths compared with turf-cut, unlimed heaths, even after five years. Although we measured establishment after one year, the results are expected to be representative of final establishment: De Graaf *et al.* (1998) observed the highest mortality in the first year after sowing.

Since turf-cutting had such a pronounced effect on germination and establishment it was interesting to see to what extent soil parameters changed after turf-cutting and how this varied with turf-cutting depth. Organic matter is known to contain high amounts of humic substances both in soluble and insoluble form (e.g. Beyer, 1996; Wilcken *et al.*, 1997). Soluble and insoluble organic compounds include humic substances that can complex and detoxify aluminium (Cronan *et al.*, 1978; Manley *et al.*, 1987; Anderson, 1991; Tomohiro and Bell, 1992; Ritchie 1995; Beyer, 1996). The soluble organic compounds include humic and fulvic acids (Currie *et al.*, 1996; Nasholm *et al.*, 1998). The DOC content is considered to be an estimate of the amounts of humic acids present (Impellitteri *et al.*, 2002). In addition, humic acids were found to colour the soil extracts strongly. A highly significant linear correlation between humic acid concentrations and the light absorption was observed. This allowed the light absorption values of the soil extracts to be used as efficient indicators of the humic acids content. Both DOC and light absorption values suggested a severe decrease in humic acid content with turf-cutting depth, a decrease which was clearly related to the amount of organic material removed.

Previous research (de Graaf *et al.*, 1997, 1998) suggested the detrimental effect of aluminium on the seed germination and establishment of herbaceous species. This can have a major effect, especially on the heathland soils that suffer from acidification. Kinraide (1997) observed increased phytotoxic Al^{3+} concentrations below pH 4.5. Detoxification of phytotoxic aluminium is therefore highly important in these systems. Van Hees *et al.* (2000, 2001) showed a strong relation between soil

pH and Al^{3+} activity in mineral soils, whereas complexation between Al^{3+} and organic compounds was observed in the organic layer. As shown in **figure 5**, aluminium concentrations in the topsoil (organic) were significantly higher than those in plots cut down to a deeper level. Although different aluminium fractions are responsible for different processes (e.g. toxicity) in the soil, practical problems prevented us from analysing the individual aluminium fractions. Aluminium was measured using an ICP after soil extractions with bi-distilled water and 0.2 M NaCl, which means that all dissolved fractions containing aluminium contributed to the total levels measured in the samples, including aluminium bound to dissolved humic substances.

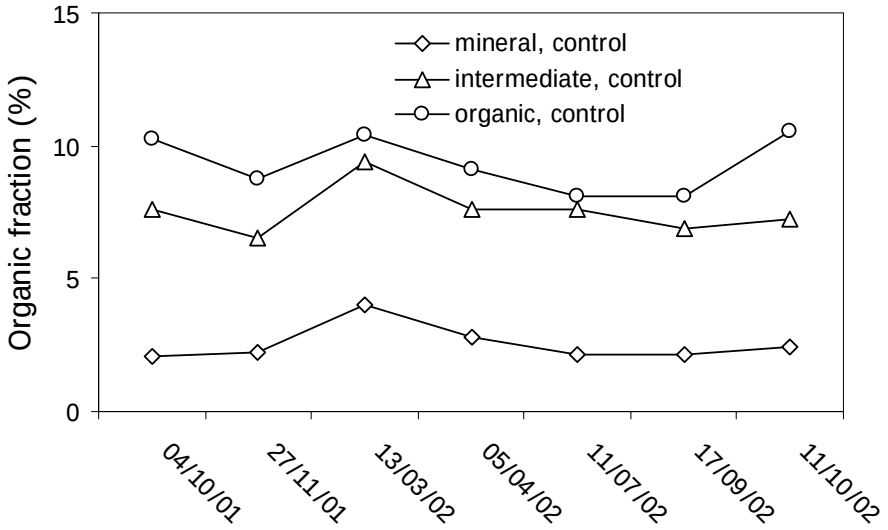

Figure 2: Mean organic fraction percentages during the first year after sowing. Turf-cutting was done at three depths ('organic', 'intermediate' and 'mineral').

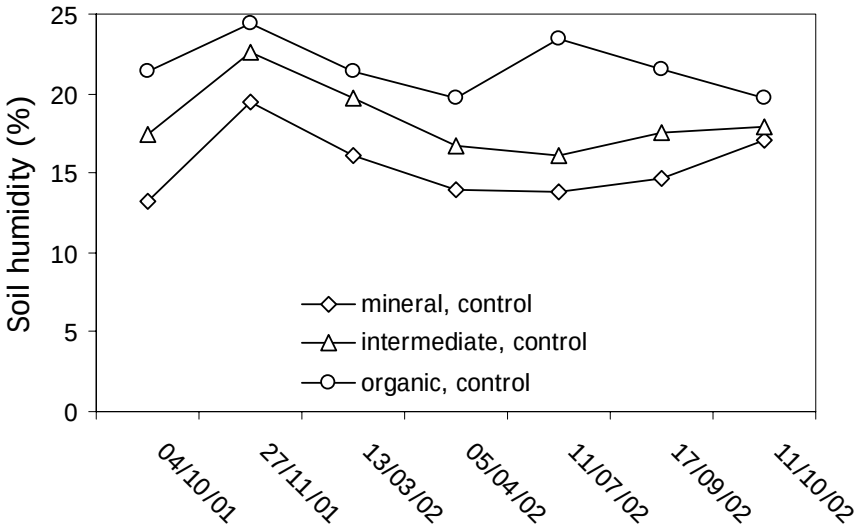

Figure 3: Mean soil humidity percentages during the first year after sowing. Turf-cutting was done at three depths ('organic', 'intermediate' and 'mineral').

In addition to detoxification of aluminium by complexation to humic substances, many authors have reported detoxification by base cations such as calcium (Korcak, 1990; Ryan *et al.*, 1994). This is in line with the higher germination and establishment in plots that contained more organic material, and thus humic acids, and with the higher germination and establishment in plots that received additional calcium in the form of lime.
It is clear that turf-cutting causes numerous changes in physical and chemical parameters. Soil humidity, pH and nutrient concentrations changed significantly after turf-cutting. The removal of the organic layer, or part of it, immediately reduces the water retention capacity.

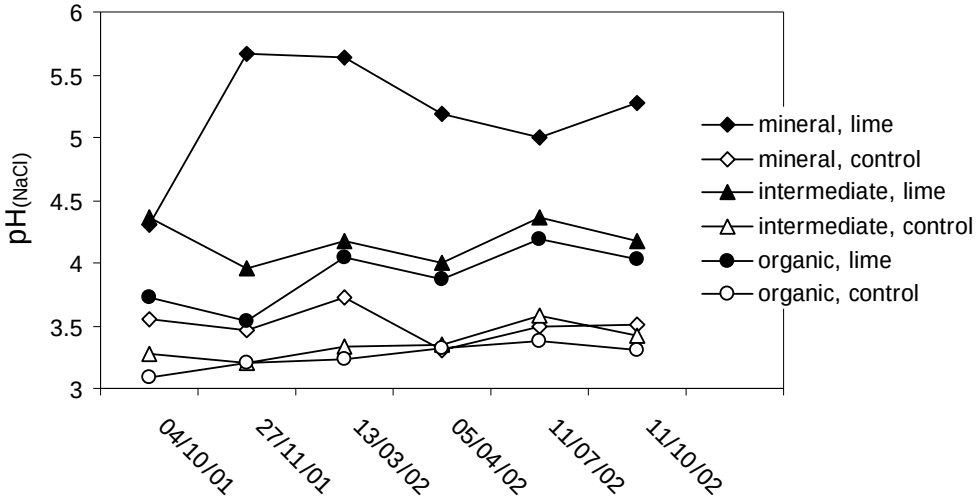

Figure 4: Mean $pH_{(NaCl)}$ values of the soil extracts during the first year after sowing. Turf-cutting was done at three depths ('organic', 'intermediate' and 'mineral'). Limed plots are presented as closed symbols, control plots are presented as open symbols.

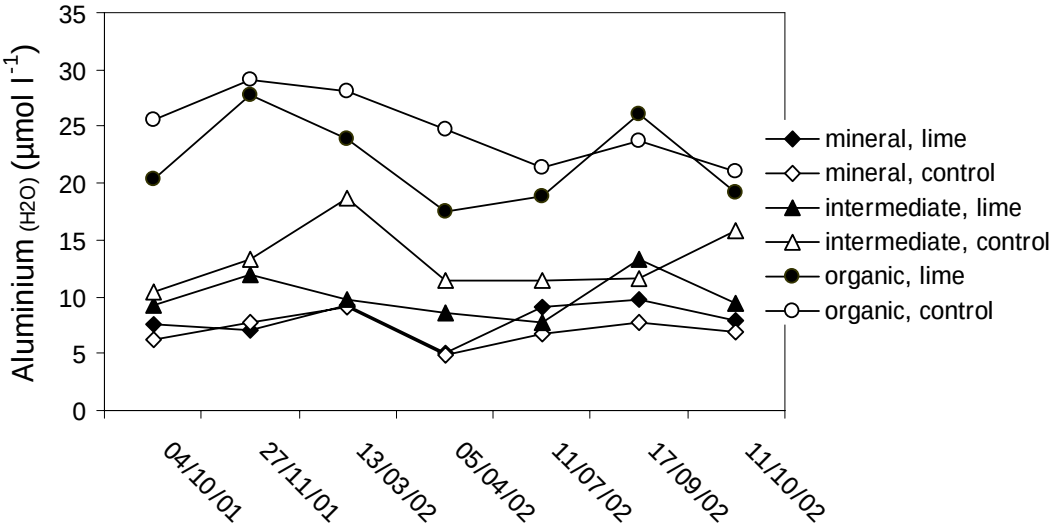

Figure 5: Aluminium concentrations of the soil extracts during the first year after sowing. Turf-cutting was done at three depths ('organic', 'intermediate' and 'mineral'). Limed plots are presented as closed symbols, control plots are presented as open symbols.

Additionally, soil pH increases after turf-cutting, since the acidified, organic top layer is removed. These factors can affect germination.Therefore, the results of the field experiments did not enable us to decide whether aluminium toxicity and the detoxifying effects of calcium and/or humic acids are the main driving forces of germination and establishment after turf-cutting. Hence, a germination experiment was performed under controlled conditions in which pH, nutrients, temperature, light and moisture content were carefully controlled. The results of this experiment support those of the field experiment, in that increased aluminium concentrations were found to reduce germination and establishment. Addition of lime and/or humic acids resulted in higher germination and establishment and reduced the negative effects of aluminium. It is likely that this was the result of the detoxifying effect on aluminium by humic acids and calcium. Aluminium can reduce the uptake of minerals, particularly divalent cations such as calcium (Foy *et al.*, 1978). However, addition of Ca in all treatments made it very unlikely that the decreased germination was due to the reduced Ca uptake. Since the levels of humic acids in the field experiment were higher in the 'organic' and 'intermediate' plots, it is likely that the higher germination rates in these plots resulted from immobilizng of aluminium by humic acids.

Turf cutting as a management practice is widely applied, sometimes to very different vegetation types. It is successful in restoring oligotrophic systems by removing acidified and eutrophied material. Turf cutting also results in the removal of a large part of the viable seed bank, which, depending on the history of the heathland, will contain much of the more aggressive species such as grasses.

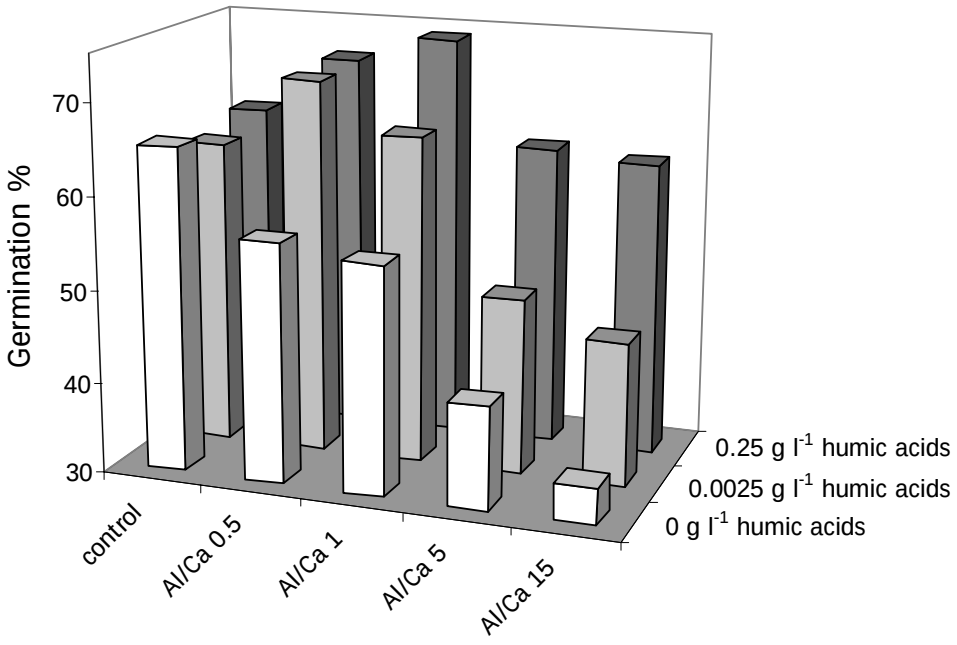

Figure 6: Mean germination percentages of *Arnica montana* in different treatments. Al/Ca ratios were applied as 0, 0.5, 1, 5, and 15. Humic acids were added in the concentrations 0, 0.0025, and 0.25 g l^{-1}. The 0 g l^{-1} humic acid concentration served as a control for the humic acid treatments, whereas the Al/Ca 0 ratio served as a control for the Al/Ca ratio treatments.

Table 4: Mean values **(a)** and results of a general linear model procedure **(b)** of Al/Ca ratio and humic acid effect on the germination and germination rate of seeds of *Arnica montana*. All variables were log-transformed.

(a) Mean values

	Low concentration humic acids (0 g l^{-1})					Intermediate concentrations humic acids (0.0025 g l^{-1})					High concentrations humic acids (0.25 g l^{-1})				
	Al/Ca					Al/Ca					Al/Ca				
	0	0.5	1	5	15	0	0.5	1	5	15	0	0.5	1	5	15
Germination (%)	65	56	55	41	34	63	71	66	49	46	65	71	74	63	63
Germination rate (seeds day^{-1})	8.29	7.26	6.74	5.09	4.34	7.14	7.89	7.77	6.17	5.54	7.77	7.31	7.6	7.26	7.71

(b) Statistical results

	Al/Ca ratio effect				Humic acid effect				Al/Ca*humic acid interaction effect			
	d.f.	*MS*	*F*	*p-value*	*d.f.*	*MS*	*F*	*p-value*	*d.f.*	*MS*	*F*	*p-value*
Germination (%)	4	294.985	20.547	**0.000**	2	474.614	33.058	**0.000**	8	47.177	3.286	**0.003**
Germination rate (seeds day^{-1})	4	2.534	6.788	**0.000**	2	2.724	7.297	**0.001**	8	0.893	2.391	**0.025**

Thus, turf-cutting might alter competition between seeds; it can be beneficial for heathland species in terms of germination and establishment. However, almost 90% of the seeds of heathland species are located in the topsoil (Putwain and Gillham, 1990) and are thus removed as well. On top of that, many rare herbaceous species do not form long-term, persistent seed banks (Bakker *et al.*, 1996; Bekker *et al.*, 1998) and it is likely that these species disappeared from the seed bank of grass dominated heathlands (even after turf-cutting). Germination and establishment is of vital importance for the survival of these species and many of these species are depending on the presence of remaining populations and their dispersal capacity. In isolated areas where these species became extinct (no viable seeds in the seed bank), recolonisation is not likely to occur. In these situations additional restoration managements such as reintroduction can be considered in order to restore diversity.
After turf removal, additional liming appeared to be a prerequisite to achieve successful germination and establishment. Moreover, turf-cutting has major effects on soil characteristics, both physical and chemical. The present study found a clear aluminium toxicity effect, resulting in decreased germination and establishment of *A. montana*. However, an effect of moisture content and amounts of nutrients in the organic layer could not be excluded and may co-occur with effects of liming and detoxification by humic acids. Summarizing, the herbaceous species are vulnerable to poor soil conditions directly after turf-cutting. It is therefore important that restoration of species-rich heathlands and species-rich grasslands is carefully done. Knowledge of the chemical and physical background is highly important in order to apply specific restoration measures.

Acknowledgements

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References

Aerts, R., Heil, G.W. 1993. *Heathlands: patterns and processes in a changing environment*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Anderson, J.M. 1991. The effects of climate change on decomposition processes in grasslands and coniferous forests. *Ecological Applications*, 1: 326-347.

Bakker, J.P., Podschlod, P., Strykstra, R.J., Bekker, R.M., Thompson, K. 1996. Seed banks and seed dispersal: important topics in restoration ecology. *Acta Botanica Neerlandica*, 45: 461-490.

Bekker, R.M., Schaminée, J.H.J., Bakker, J.P., Thompson, K. 1998. Seed bank characteristics of Dutch plant communities. *Acta Botanica Neerlandica*, 47: 15-26.

Berendse, F. 1990. Organic matter accumulation and nitrogen mineralization during secondary succession in heathland ecosystems. *Journal of Ecology*, 78: 413-427.

Beyer, L. 1996. Soil organic matter composition of spodic horizons in Podzols of Northwest German Lower Plain. *The Science of the Total Environment*, 181: 167-180.

Bobbink, R., Boxman, A.W., Fremstad, E., Heil, G., Houdijk, A.L.F.M., Roelofs, J.G.M. 1992. Critical loads for nitrogen eutrophication of terrestrial and wetland ecosystems based upon changes in vegetation and fauna. In: Grennfelt, P., Thörnelöf, E. (Eds.). *Critical loads for nitrogen*. Nordic Council of Ministers, Copenhagen, Denmark. pp. 111-159.

Bobbink, R., Hornung, M., Roelofs, J.G.M. 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, 86: 717-738.

Cronan, C.S., Reiners, W.A., Reynolds, R.C.Jr., Lang, G.E. 1978. Forest floor leaching: contributions from mineral, organic and carbonic acids in New Hampshire subalpine forests. *Science*, 200: 309-311.

Currie, W.S., Aber, J.D., McDowell, W.H., Boone, R.D., Magill, A.H. 1996. Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. *Biogeochemistry*, 35: 471-505.

De Graaf, M.C.C., Bobbink, R., Verbeek, P.J.M., Roelofs, J.G.M. 1997. Aluminium toxicity and tolerance in three heathland species. *Water, Air and Soil Pollution*, 98: 229-239.

De Graaf, M.C.C., Verbeek, P.J.M., Bobbink, R., Roelofs, J.G.M. 1998. Restoration of species-rich dry heaths: the importance of appropriate soil conditions. *Acta Botanica Neerlandica*, 47: 89-113

Diemont, W.H. 1994. Effects of removal of organic matter on productivity in heathlands. *Journal of Vegetation Science*, 1: 129-132.

Fennema, F. 1992. SO_2 and NH_3 deposition as possible cause for the extinction of *Arnica montana* L. *Water, Air and Soil Pollution*, 62: 325-336.

Ferguson, I.K. 1976. *Arnica* L. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.). *Flora Europaea*. Cambridge University Press, Cambridge, United Kingdom, pp. 189-190.

Foy, C.D., Chaney, R.L., White, M.C. 1978. The physiology of metal toxicity in plants. *Annual Reviews of Plant Physiology*, 29: 511-566.

Heil, G.W., Diemont, W.H. 1983. Raised nutrient levels change heathland into grassland. *Vegetatio*, 53: 113-120.

Houdijk, A.L.F.M., Verbeek, P.J.M., Van Dijk, H.F.G., Roelofs, J.G.M. 1993. Distribution and decline of endangered heathland species in relation to the chemical composition of the soil. *Plant and Soil*, 148: 137-143.

Imperllitteri, C.A, Yuefeng, L., Saxe, J.K., Allen, H.E., Peijenburg, W.J.G.M. 2002. Correlation of the partitioning of dissolved organic matter fractions with the desorption of Cd, Cu, Ni, Pb and Zn from 18 Dutch soils. *Environment International*, 28: 401-410.

Kinraide, T.B. 1997. Reconsidering the rhizotoxicity of hydroxyl, sulphate and fluoride complexes of aluminium. *Journal of Experimental Botany*, 48: 1115-1124.

Korcak, R.F. 1990. Calcium protects against aluminium toxicity. In: Van Beusichem, M.L. (Ed.). *Plant nutrition - Physiology and applications*. Kluwer Academic Publishers, pp. 409-412.

Lee, J.A., Caporn, S.J.M. 1998. Ecological effects of atmospheric reactive nitrogen deposition on semi-natural terrestrial ecosystems. *New Phytologist*, 139: 127-134.

Manley, E.P., Chesworth, W. and Evans, L.J. 1987. The solution chemistry of podzolic soils from the eastern Canadian shield: a thermodynamic interpretation of the mineral phases controlling soluble Al^{3+} and H_4SiO_4. *Journal of Soil Science*, 38: 39-51.

Nasholm, T., Ekblad, A., Nordin, A., Giesler, R., Hogberg, M. and Hogberg, P. 1998. Boreal forest plants take up organic nitrogen. *Nature*, 392: 914-916.

Putwain, P.D., Gillham, D.A. 1990. The significance of the dormant viable seed bank in the restoration of heathlands. *Biological Conservation*, 52: 1-16.

Ritchie, G.S.P. 1995. Soluble aluminium in acidic soils: Principles and practicalities. *Plant and*

Soil, 171: 17-27.

Ryan, P.R., Kinraide, T.B., Kochian, L.V. 1994. Al^{3+}-Ca^{2+} interactions in aluminium rhizotoxicity. I. Inhibition of root growth is not caused by reduction of calcium uptake. *Planta*, 192: 98-103.

Scheffer, F., Schachtschabel, P. 1979. *Lehrbuch der Bodenkunde* . Ferdinand Enke Verlag, Stuttgart, Germany.

Snow, C.S.R., Marrs, R.H. 1997. Restoration of *Calluna* heathland on a Bracken *Pteridium* - infested site in North West England. *Biological Conservation*, 81: 35-42.

Tomohiro, B., Bell, C. 1992. Soil solid and solution phase changes and mung bean response during amelioration of aluminium toxicity with organic matter. *Plant and Soil*, 140: 183-196.

Ulrich, B. 1983. Soil acidity and its relation to acid deposition. In: Ulrich, B., Pankrath, J. (Eds.). *Effects of accumulation of air pollutants in forest ecosystems*. Reidel Publishing Compagny, Dordrecht, The Netherlands, pp. 127-146.

Ulrich, B. 1991. An ecosystem approach to soil acidification. In: Ulrich, B., Summer, M.E. (Eds.). *Soil acidity*. Springer Verlag, Berlin, Germany, pp. 28-79.

Van Breemen, N., Burrough, P.A., Velthorst, E.J., Van Dobben, H.F., De Wit, T., Ridder, T.B., Reijnders, H.F.R. 1982. Soil acidification from atmospheric ammonium sulphate in forest canopy throughfall. *Nature*, 299: 548-550.

Van Dam, D., Van Dobben, H.F., Ter Braak, C.F.J., De Wit, T. 1986. Air pollution as a possible cause for the decline of some phanerogamic species in the Netherlands. *Vegetatio*, 65: 47-52.

Van Hees, P.A.W., Lundström, U.S., Starr, M, Giesler, R. 2000. Factors influencing aluminium concentrations in soil solution from podzols. *Geoderma*, 94: 289-310.

Van Hees, P., Lundström, U., Danielsson, R., Nyberg, L. 2001. Controlling mechanisms of aluminium in soil solution - an evaluation of 180 podzolic forest soils. *Chemosphere*, 45: 1091-1101.

Werger, M.J.A., Prentice, I.C., Helsper, H.P.H. 1985. The effect of turf-cutting to different depths on Calluna heathland regeneration. *Journal of Environmental Management*, 20: 181-188.

Wilcken, H., Sorge, C., Schulten, H.-R. 1997. Molecular composition and chemometric differentiation and classification of soil organic matter in podzol B-horizons. *Geoderma*, 76: 193-219.

Chapter 7

GENERAL DISCUSSION AND SYNTHESIS

Leon J.L. van den Berg

General discussion and synthesis

Direct and indirect effects of atmospheric S and N deposition have resulted in the decline, or even disappearance, of many natural and semi-natural ecosystems, as a result of acidification and eutrophication of the soil (see, e.g., Heil and Diemont, 1983; Bobbink *et al.,* 1998). Great biodiversity losses have been reported, since the atmospheric deposition of these acidifying and eutrophying compounds started to increase in the first half of the 20th century. It were especially those vegetation types typically found on slightly buffered and/or acidic soils, which proved sensitive and declined rapidly in terms of both area and biodiversity. In the Netherlands, these vulnerable vegetation types can be found on oligotrophic and mesotrophic soils and include heathlands, species-rich acid grasslands, mat grass swards and fen systems.
A decline in species diversity and a deterioration of habitats have often been demonstrated in acid and acid-sensitive ecosystems in areas with a high NH_4^+ deposition rate (e.g. The Netherlands), indicating an association between increased NH_4^+ concentrations and the decline of many characteristic herbaceous species. Restoration measures developed in the 1980s and 1990s were applied to counteract the detrimental effects of acidification and eutrophication and to restore the vegetation. Among these, sod cutting and the addition of lime are widely applied measures, which successfully removes the excess eutrophied material and restore soil pH, respectively (see, e.g., Gimmingham, 1972; Jansen and Roelofs, 1996; Kreutzer, 1995; Roelofs *et al.*, 1996; Webb, 1998).
This thesis focuses on the changes in species composition due to different forms of N in N deposition and its interaction with biogeochemical parameters. Our research examined the effects of N deposition on acid and acid-sensitive grassland and heathland ecosystems and more specifically the effects of NH_4^+ deposition on the vegetation development. We also investigated the effects of restoration measuressuch as sod cutting and additional liming on soil chemistry, microbial activity and plant development.

N deposition effects on vegetation development in dune grasslands

Experiments with manipulating N supplies have shown that increased N deposition rates result in an increased growth of competitive species (mainly grasses) at the expense of characteristic (mainly herbaceous species) species (Bobbink, 1991; Carroll *et al.,* 2003). Our results are consistent with this observation, as the biomass and cover percentage of fast growing graminoid species, e.g. *Calamagrostis epigejos* and *Carex arenaria*, were found to increase drastically with increasing N deposition rates (**chapter 2**). Consequently, characteristic key species such as *Galium verum* and *Carlina vulgaris* started to decline and disappeared (**chapter 2**). N manipulation experiments at low background N deposition such as those described in **chapter 2** are extremely valuable for the establishment of empirical critical loads for N deposition. Critical loads are used to design a sound policy and management measures to ensure vegetation conservation. Such experiments however, are expensive and have rarely been done. **Chapter 2** shows that increased N deposition rates resulted in grass encroachment at deposition rates of 20 kg N ha^{-1} yr^{-1}. This is

consistent with the findings of several other studies and field observations, as many species-rich grasslands and stable dune grasslands become dominated by monocultures of grass in areas where N deposition exceeds 20 kg N ha^{-1} yr^{-1} (Jones *et al.*, 2002, 2003; Bobbink *et al.,* 2003).
In situations of increased N availability, ecosystems may shift from an N-limited to a P-limited status (Falkengren-Grerup and Diekmann, 2003). Kooijman and Besse (2002), Morecroft *et al.* (1994) and Carroll *et al.* (2003) did indeed find P limitation in areas affected by high atmospheric N inputs. Our research showed that the total amount of N stored in the vegetation increased significantly with elevated N depositions levels and we found that the growth of most graminoid species in stable dunes increased with increasing N loads, indicating that stable dunes were N-limited (**chapter 2**). N limitation has often been shown in heathlands (Boorman and Fuller, 1982; Bobbink *et al.*, 1988; Bobbink, 1991; Süß *et al.*, 2004) and strong evidence for N limitation has been found in dune grasslands, even at high (>100 kg N ha-1 yr^{-1}) N loads (Willis, 1963). In addition, Koerselman and Meuleman (1996) observed a critical N/P ratio of 16 above which P limitation rather than N limitation might be expected. However, we found N limitation when N/P ratios higher than 16 at elevated N loads for the graminoid species *C. epigejos* and *C. arenaria* (**chapter 2**). These results are in line with an earlier study showing N limitation at high N/P ratios (from 29 up to 35) in *Brachypodium pinnatum* after three years of N fertilisation in calcareous grasslands (Bobbink, 1991). Higher N/P ratios have commonly been found in grass species (Güsewell, 2004) and it is, therefore, suggested that grass species are able to benefit more from N enrichment than other (herbaceous) species, due to their ability to withstand relatively low concentrations of P (Bobbink, 1991; Güsewell, 2004; **this thesis**). The research reported on in **chapter 2** showed N/P ratios in *G. verum* plants to be within the range of N limitation, rather than P limitation. We therefore assume that the decline of this herbaceous species is caused by competition for light, rather than limitation of P.
Our results support the idea that increased N availability, as a result of increased atmospheric N deposition, can be a major cause of the decline of species-rich grasslands (Aerts and Heil, 1993; Roelofs *et al.,* 1996; Lee and Caporn, 1998; Bobbink *et al.,* 1998). However, the decline of herbaceous species cannot be primarily attributed to P limitation, or base cation deficiency, but is mostly due to increased competition for light or direct NH_4^+ toxicity.

Effects of NH_4^+ on plants and vegetation development

Atmospheric N deposition has not only an eutrophying effect but also an acidifying effect. Most of the atmospheric N in Western Europe is deposited in reduced form (NH_3 and NH_4^+). Both an increased N deposition and a reduced nitrification rate, due to acidification, contribute directly, or indirectly, to increased NH_4^+ concentrations. This may eventually lead to a shift in the dominant form of mineral N (from NO_3^- to NH_4^+) and increased NH_4^+/NO_3^- ratios in the soil. NH_4^+ is therefore the common form of inorganic N at low soil pH (Gigon and Rorison, 1972; Troelstra *et al.*, 1990). In degraded acidified heathlands soil solution pH range from 3.8 to 4.5 and NH_4^+ concentrations range from 300 to 650 µmol kg^{-1} DW (de Graaf *et al.,* 1994; Roelofs *et*

al., 1996; Dorland *et al.,* 2003). It has been shown that such elevated NH_4^+ concentrations have detrimental effects on the germination, growth and survival of characteristic plant species (Mehrer and Mohr, 1989; De Graaf *et al.,* 1998a; Dorland *et al.,* 2003; Vergeer *et al.,* 2003; **chapter 3** of this thesis). From our hydroponic study it became clear that the negative effects of elevated NH_4^+ concentrations were aggravated by acidic conditions (**chapter 3**). The growth and survival of acid-sensitive rare and endangered species *Antennaria dioica* and *Succisa pratensis* were negatively affected by high NH_4^+ concentrations. These effects were even more pronounced at low pH (**chapter 3**). In contrast, acid-tolerant species such as the grass *D. flexuosa* proved tolerant to elevated NH_4^+ concentrations at low pH levels. This suggests that such species, which can naturally be found on slightly acidic to acidic soils, are adapted to NH_4^+ nutrition. This is in line with results from several other studies, in which acid-tolerant species were shown to tolerate high NH_4^+ concentrations (Gigon and Rorison, 1972; Falkengren-Grerup and Lakkenborg-Kristensen, 1994; Falkengren-Grerup, 1995) and might explain why these species are not affected by elevated NH_4^+ concentrations. In a comparative study by Smart *et al.* (2004) clear correlations were found between the form of N deposited from the atmosphere and changes in the vegetation of grasslands, heathlands and bogs. They found that reduced N explained significant changes in grassland vegetation, whereas no such correlations could be found for oxidised N, strongly suggesting that the ratio of reduced and oxidised N affects the vegetation composition. These results are in accordance with our large mesocosm experiment, which we performed on nutrient-poor heathland soils (**chapter 4**). In this experiment the grass species *D. flexuosa* and *Nardus stricta* grew equally well at both high and low NH_4^+/NO_3^- ratios in the soil and in limed, as well as control (unlimed) situations. There was, however, a clear decline in the presence and coverage of the herbaceous species at elevated NH_4^+/NO_3^- ratios.

At higher ratios, increased shoot biomass was found for the grass species *D. flexuosa*. This was also found in stable dune grasslands for *C. epigejos* (**chapter 2**) at elevated N concentrations (applied as NH_4^+/NO_3^-). Investment in above-ground biomass, that is, increasing the shoot/root ratio, resulted in a high competitive vigour for light. Both *C. epigejos* and *D. flexuosa* are considered to be typically responsible for severe grass encroachment and our experiments show that they are able to do so by increasing shoot length and coverage, because they use N sources efficiently, regardless of the form of N available, even at low P availability.

Acid-sensitive species such as *A. dioica, Arnica montana, Gentiana pneumonanthe and Danthonia decumbens*, declined in our mesocosm experiments when grown at high NH_4^+/NO_3^- ratios. When grown in hydroculture, *A. dioica* was found to be sensitive to high NH_4^+ concentration in combination with low pH (**chapter 3**). This might apply to the grass *Danthonia decumbens* as well. As a result of elevated NH_4^+/NO_3^- ratios in the soil, we found that the coverage of most herbaceous species decreased and observed yellow leaves and stunted roots in *A. montana*, *G. pneumonanthe* and *D. decumbens* (**chapter 4**), indicating that these species suffered from an NH_4^+ toxicity syndrome (Mehrer and Mohr, 1989). For the shrub *Calluna vulgaris* poor growth was found in non-limed mesocosms at high NH_4^+/NO_3^- ratios (**chapter 4**). These results

were corroborated by those of the hydroponic experiment discussed in **chapter 3**, which showed that *Calluna vulgaris* exhibited low biomass and chlorosis at high NH_4^+ concentrations (1000 µmol l^{-1}) in combination with a low pH (3.5). This might suggest that, in addition to the effects of grass encroachment and beetle outbreaks (Roelofs, 1986; Aerts *et al.*, 1990; Bobbink and Heil, 1993), negative effects of high NH_4^+ concentrations and NH_4^+/NO_3^- ratios can contribute to the decline of *Calluna vulgaris* in Western European heathlands suffering from acidification and very high NH_4^+ concentrations.
Although few studies have focussed on the effects of different atmospheric NH_4^+ and NO_3^- depositions, the need for such studies has been widely recognised (Bobbink *et al.*, 1998; Bobbink *et al.*, 2003; Smart *et al.*, 2004). The results reported in this thesis clearly indicate that at fixed N deposition rates, the NH_4^+/NO_3^- ratio can markedly affect the vegetation composition of nutrient-poor and acidified grasslands and heathlands. At high ratios, acid-sensitive herbaceous species decline at the expense of fast-growing grass species. This might be due to increased competition for light with the fast growing grass species, as well as to direct NH_4^+ effects.

Effect of NH_4^+ nutrition on nutrient status

In addition to reduced plant performance, in terms of reduced growth or increased mortality, cation deficiency has been suggested as a major NH_4^+ toxicity mechanism, as NH_4^+ uptake results in a decrease in cation uptake (Kirkby, 1968; Van Beusichem *et al.*, 1988). De Graaf *et al.* (1998a) showed Ca and Mg deficiency in *Cirsium dissectum*, when grown at high NH_4^+ concentrations and low pH. Lucassen *et al.* (2003) found similar results for *C. dissectum* when grown at both high and low pH and concluded that NH_4^+ was responsible for the reduced cation content. In the research reported on in this thesis, we observed a reduced cation content in the acid-tolerant species *Calluna vulgaris* and *D. flexuosa* and the supposed slightly acid-tolerant *G. pneumonanthe*, when grown in hydroculture at high NH_4^+ concentration. Strikingly, these species did not show reduced performance at high NH_4^+ concentrations. In addition, a decrease in external pH resulted in a decreased uptake of cations and, hence, reduced Ca, Mg and K concentrations in the roots and shoots of *G. pneumonanthe*. The same effect was observed for the cation concentrations in the shoots of *Calluna vulgaris*. This illustrates that a combination of low pH and high NH_4^+ concentrations can lead to serious deficiencies in time, even for supposedly acid-tolerant species. These results are consistent with those of earlier studies, in which a decrease in the pH of the rhizosphere and apoplast was found to decrease cation uptake, resulting in cation deficiencies in the plant (Van Beusichem *et al.*, 1988; Boxman *et al.*, 1991; Marschner, 1995). Additionally, internal acidification of acid-sensitive plants due to NH_4^+ uptake has been suggested as another mechanism for NH_4^+ toxicity (Gerendás *et al.*, 1990; Lucassen *et al.*, 2003). However, experiments including both NH_4^+-sensitive and NH_4^+-tolerant species have demonstrated that most species showed a clear decrease in pH upon NH_4^+ nutrition (Paulissen *et al.*, 2004; **chapter 3** of this thesis), indicating that base cation deficiency is most likely to be the determinant mechanism of NH_4^+ toxicity in herbaceous species.

To prevent NH_4^+ toxicity many plants respond to NH_4^+ uptake by synthesising specific amino acids and amines, assimilating NH_4^+ into amino acids with a low C/N ratio such as arginine (C/N ratio 6/4) and asparaginine (C/N ratio 4/2; Marschner, 1995). Our research found that these N-rich amino acids greatly increased in plants grown at high NH_4^+ concentrations (**chapter 4**). Such an increase in free amino acid concentrations in response to high N loads has also been found in other studies (see, e.g., Van Dijk and Roelofs, 1988; Thomassen *et al.,* 2003). The increased concentrations of arginine, asparaginine and serine found when *D. flexuosa*, *G. pneumonanthe* and *A. montana* were grown at high NH_4^+/NO_3^- ratios indicate that these plants need to produce larger quantities of free amino acids to detoxify the large quantities of NH_4^+. The ability to produce the free amino acids to detoxify NH_4^+ may be species-specific and determine NH_4^+ toxicity effects. It is not unlikely that the grass *D. flexuosa* has a greater ability to do this than supposedly NH_4^+-sensitive species such as *G. pneumonanthe* and *A. montana*.

Decline of heathlands and species-rich grasslands

In **chapter 2** it was shown that the biomass of the grass species *C. epigejos* and *C. arenaria* increased at elevated N loads in stable dune grassland mesocosms. In addition, the herbaceous species *G. verum* and *Carlina vulgaris* showed a major decrease in total biomass after two years at elevated N loads. Competition for light and nutrients with the graminoid species was therefore assumed to be the most important factor determining the decrease and decline of *G. verum*, rather than toxic or deficiency effects. Competition for light and nutrients may also be an important determining factor in heathlands (Bobbink, 1991). *D. flexuosa* is a highly competitive, fast-growing species, which can overgrow small, herbaceous species. This might explain the decline of many herbaceous species in heathlands in Western Europe and their replacement by competitive grasses such as *D. flexuosa*. *N. stricta*, on the other hand, is not a fast-growing competitor but it can form dense swards and thereby prevent herbaceous species from germinating and establishing. Our results are consistent with those of studies by Heil and Diemont (1983) and Aerts and Heil (1993), who found a shift in dominance from dwarf shrub vegetation (e.g. *Calluna vulgaris* and *Erica tetralix*) towards grass-dominated vegetation (e.g. *D. flexuosa* and *Molinia caerulea*) as a result of increased N availability and increased NH_4^+ deposition. It should be noted that a direct shift from heathland vegetation towards grass vegetation was only observed in relatively young (< 5 yr), open heathland vegetation. Older heathland vegetation has a closed canopy and in such conditions heather is well able to compete with the grass species, even at high N loads. Only after canopy opening can grasses establish and out-compete heathland vegetation. At elevated N deposition however, canopy opening is very likely, as such deposition increases the risk of damage and thus canopy opening, due to outbreaks of heather beetle (*Lochmaea suturalis*; Aerts and Heil, 1993; Bobbink and Heil, 1993), winter frost, or summer drought (Power *et al.*, 1998).
However, the results reported in this thesis show that characteristic rare herbaceous species are threatened by other factors as well. Our research found the herbaceous species *A. dioica, A. montana* and *G. pneumonanthe* to be sensitive to high NH_4^+/NO_3^-

ratios, with mortality increasing with rising NH_4^+/NO_3^- ratios in the soil. As discussed in **chapter 3**, we found *A. dioica* to be sensitive to high NH_4^+ concentrations (> 500 μmol l^{-1}) in combination with a low pH (3.5). The results reported in **chapter 4** support this, as our experiments found increased survival and biomass after lime addition. The decline of *A. dioica* in species-rich grasslands in the Netherlands can therefore be explained by negative effects (direct or indirect) of acidification and/or NH_4^+ toxicity at low pH levels. Although the survival of *G. pneumonanthe* did not differ from that of *Calluna vulgaris* and *D. flexuosa* in different NH_4^+ and pH treatments (**chapter 3**), negative effects of acidification on biomass were observed. This agrees with field data showing that *G. pneumonanthe* can be regarded as more acid-sensitive than *Calluna vulgaris* and *D. flexuosa*. In contrast, *G. pneumonanthe* did show higher mortality rates after two years, when NH_4^+/NO_3^- ratios in the soil increased (**chapter 4**). As the hydroponic study covered only a short period, the sensitivity of *G. pneumonanthe* to high NH_4^+ levels may well show an accumulative effect in time. Earlier studies have found that Al concentrations increase with increasing acidification as a result of a shift in the soil buffer range (Scheffer and Schachtschabel, 1979; Van Breemen *et al.,* 1982) and it has been suggested that these high Al concentrations cause severe toxicity, resulting in the decline of many herbaceous species (Kinraide, 1997). This was illustrated by De Graaf *et al.* (1997), who showed that the acid-sensitive species *A. montana* was highly sensitive to increased Al/Ca ratios in combination with low nutrient levels. Germination experiments with *A. montana* using humic acids to buffer against high Al concentrations support this (**chapter 6**). In the experiments reported on in **chapters 3 and 4**, the plants were grown at low Al concentrations. This might explain the relatively good performance and low mortality rates of both *G. pneumonanthe* and *A. montana* at elevated NH_4^+ concentrations and NH_4^+/NO_3^- ratios. In field situations however, high Al concentrations and high NH_4^+ concentrations are commonly found together in slightly acid soil conditions. In these situations, combined with low base cation concentrations, Al may well affect the performance of many acid-sensitive species such as *A. dioica*, *G. pneumonanthe*, *S. pratensis* and *A. montana* and may contribute more to the decline of these species.

Restoration measures

Measures to restore species-rich grasslands and heathlands have been increasingly applied since the early 1980s. Removal of the top soil layer (sod cutting) was used to remove excess nutrients from the system (Bakker, 1989; Roelofs *et al.,* 1996; Webb, 1998). This method also proved successful in reducing the presence of grasses in the system and creating opportunities for the establishment of characteristic species (Snow and Marrs, 1997; De Graaf *et al.,* 1998b; Britton *et al.,* 2000). Despite such successes, poor vegetation recovery after sod cutting has been recorded in many cases of ecosystems suffering from acidification and increased atmospheric N deposition. Recent studies found high NH_4^+ accumulation levels in the first 1-2 years after sod cutting. NH_4^+ accumulation was found in both dry and wet heaths and concentrations were shown to increase to values as high as 1000 μmol l^{-1} (De Graaf *et al.,* 1998b; Dorland *et al.,* 2003). This increase in NH_4^+ concentrations was attributed to

atmospheric N deposition, which mainly takes the form of NH_4^+ and to the absence of plants which take up NH_4^+. Furthermore, NH_4^+ might accumulate, because the organic material remaining in the soil after sod cutting has a relatively high N content, which enhances mineralization rates as a result of decreased C:N ratios in the organic matter (Berendse, 1990). Finally, nitrification rates are strongly reduced, as sod cutting results in a major reduction in nitrifying bacteria (**chapter 5**). Highly competitive, nitrophilic grasses can benefit from these soil conditions and increase their biomass even at high NH_4^+ concentrations, which enables them to out-compete slower growing species (Heil and Diemont, 1983; **this thesis**). However, the research reported on in this thesis found strong evidence that the decline of the herbaceous species was not only a matter of competition for light and nutrients with the grasses, but was also greatly determined by deteriorated biogeochemical conditions, resulting in poor germination, growth, survival and recovery of the vegetation. This was found even after restoration measures such as sod cutting and we suggest that additional restoration measures such as liming are a prerequisite for successful restoration of acidic species-rich grasslands and heathlands (**this thesis**).

Another problem associated with sod cutting has been identified by experiments involving the removal of the top soil layer from acidified heathlands, which showed that the soil's capacity to buffer toxic Al concentrations was greatly reduced, as humic acids were removed along with the top soil (**chapter 6**). This resulted in increased Al toxicity effects and, hence, reduced germination of characteristic species such as *A. montana*. De Graaf *et al.* (1997) found reduced growth and survival of the acid-sensitive species *A. montana* at increased Al/Ca ratios in combination with low nutrient levels. Additional measures such as the application of lime and the restoration of the original hydrology have since been applied to restore the pH and buffer capacity of the soil (Roelofs *et al.*, 1996; De Graaf *et al.*, 1998b; Dorland *et al.*, 2004, 2005). In our research the addition of lime led to higher pH values in the soil, causing nitrification rates to increase (**chapter 5**). Moreover, the Al/Ca ratios in the soil improved, reducing the detrimental effects of free Al. In **chapter 6** it was shown that lime application after sod cutting resulted in improved germination and survival of *A. montana*. Lime clearly improves the soil pH, thereby counteracting detrimental effects of acidification (see, e.g., Dorland *et al.*, 2004; **chapter 6** of this thesis). Lime addition after sod cutting also enhanced decomposition and mineralization, as pH values became more favourable for microorganisms. Although this process resulted in increased NH_4^+ concentrations, these concentrations were not very high and, in time, nitrification overruled these processes and greatly decreased NH_4^+ concentrations again (**chapter 5**). Thus, lime addition clearly improved Al/Ca ratios in the soil and successfully counteracted the detrimental NH_4^+ accumulation in the first two years after sod cutting (De Graaf *et al.*, 1997; Dorland *et al.*, 2004; **chapters 5 and 6** of this thesis).

In sod-cut heathlands and grasslands which are not treated with lime the large amounts of accumulated NH_4^+ decrease after one to two years as a result of increased NH_4^+ uptake by plants (NH_4^+-tolerant species), micro-organisms (mainly nitrifying organisms) and increased immobilisation. However, for most characteristic herbaceous species this decrease in NH_4^+ levels after two years will come too late: as

these species do not produce a persistent seed bank, germination during the first years after sod cutting is necessary. As was shown by De Graaf *et al.* (1997) and in **chapter 6**, germination and establishment of herbaceous species in acidic soil conditions with elevated NH_4^+ concentrations is poor. The accumulation of high NH_4^+ concentrations in combination with a low pH leads to poor, or even absent, seedling establishment of especially characteristic herbaceous species. Two years after sod cutting, germination and establishment of the characteristic species has become increasingly poor as the seed bank is depleted. Additionally, other species such as *D. flexuosa* and *M. caerulea* are able to establish and thus have already started to colonise these sod-cut areas.

When the characteristic species have become locally extinct, that is, when seed banks are depleted, many of these species have problems recolonising the areas, as source populations often lie far away. Dispersal of for instance *A. montana* was shown to be only a few metres per year (De Graaf, 2000). In addition, seed dispersal via large grazers was also shown to be relatively small and often not to have positive effects for the rare herbaceous species (Mouissie, 2004).

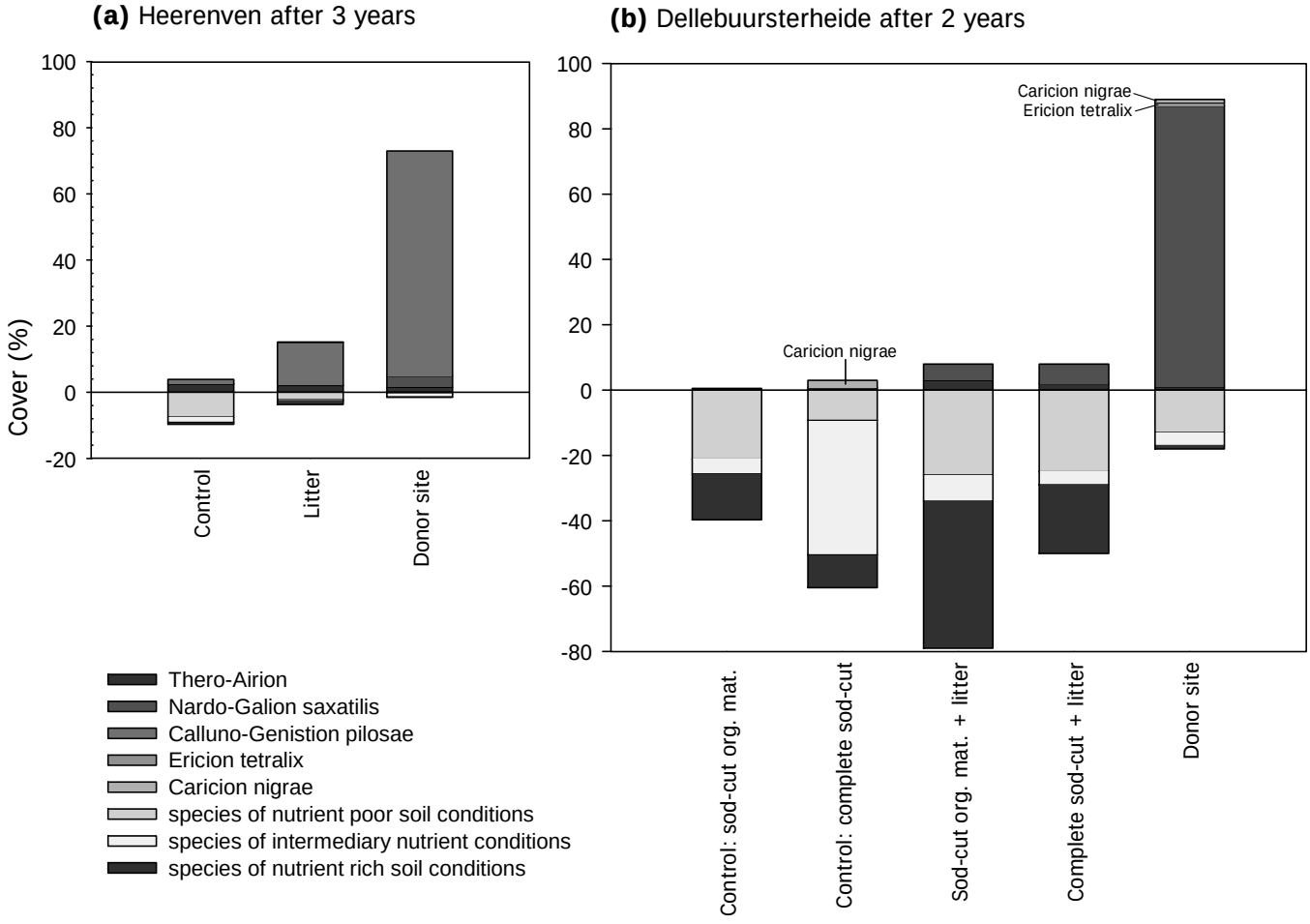


Figure 1: Cumulative cover percentage of eight different groups (five key vegetation types and three non-target species groups) at the Heerenven area (The Netherlands) after 3 years **(a)** and at the Dellebuursterheide area (The Netherlands) after 2 years **(b)**. Adapted and used with permission from Bekker *et al.* (2005).

In these situations, the reintroduction of specific characteristic key species may be a successful restoration measure, provided that the (biogeochemical) soil conditions are restored (Vergeer *et al.*, in press).
We have investigated the effects of litter application to two areas where the seed bank had been depleted, the Heerenven area (**figure 1a**) and the Dellebuursterheide area (**figure 1b**). This litter, originating from species-rich grasslands and heathlands, included large quantities of seeds which were thus introduced or reintroduced to the depleted soil. This type of restoration measure is relatively new to the Netherlands. It has until now mostly been applied on former arable soils which were converted to heathlands and hence were sod-cut to considerable depth, usually down to the mineral layer.
Figure 1 shows that the application of litter led to clear favourable results after 2 to 3 years, in terms of the development of key vegetation types (positive cover values). In addition, it resulted in a lower cover of non-key vegetation types in these areas (negative cover values). In general, *Calluna vulgaris* and *E. tetralix* developed well in the litter-treated plots and interesting species such as *C. dissectum*, *Potentilla erecta* and *Galium saxatile*, were reasonably successful after reintroduction via litter.

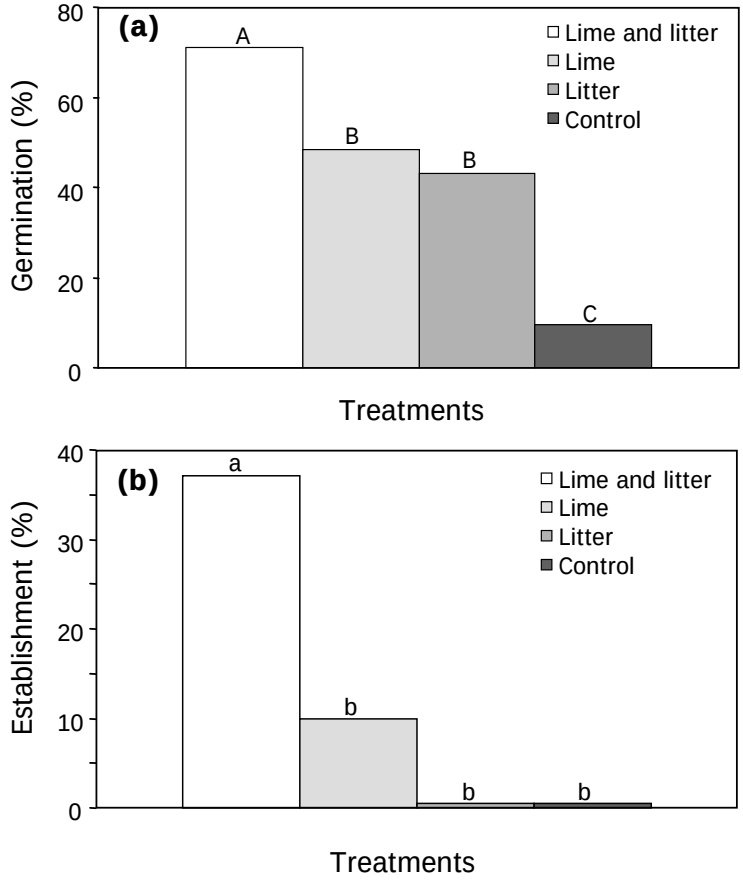


Figure 2: Mean germination (**a**) and establishment (**b**) of *Arnica montana* in a sod-cut heathland in plots treated with lime, litter, or a combination of these. Post-hoc significance differences are shown.

When litter is applied to these sod-cut areas, germination conditions for these species changes markedly. This was illustrated by a germination experiment in plots where litter (enriched with *A. montana* seeds) was applied to an acidified (pH 4) sod-cut heathland. Germination of *A. montana* (after 6 months) significantly improved in the plots to which litter was applied (**figure 2**). In addition, the application of lime as a restoration measure also improved germination and a combination of these two treatments improved germination even more. However, results in terms of establishment (after 18 months) only proved better than the controls, when the combined treatment was applied, implying that germination conditions were improved by litter, but that soil conditions determined establishment in the long term (**figure 2**). Thus, while litter may improve factors such as moisture content and micro-habitat, establishment seems to be determined mostly by soil conditions, especially soil pH and Al/Ca ratio.

The results of the research described in this thesis clearly reveal that poor soil conditions and the resulting poor development of characteristic herbaceous species can be remedied successfully. Measures such as sod cutting proved successful under the condition that they are applied in combination with additional liming in acidic conditions. It became clear that, in addition to environmental conditions, recolonisation largely depended on the availability of a viable seed bank and on the dispersal and colonisation capacities of the species (Bekker *et al.*, 1998). Many of the characteristic herbaceous species however, lack such a viable seed bank and distances from the nearest source populations have become too large for the characteristic key species to recolonise (Bakker *et al.*, 1996; Britton *et al.*, 2000; Ehrlén and van Groenendael, 2001).

REFERENCES

Aerts, R., Heil, G.W. 1993. *Heathlands: patterns and processes in a changing environment.* Kluwer Academic Publishers, Dordrecht, The Netherlands.

Aerts, R., Berendse, F., De Caluwe, H., Schmitz, M. 1990. Competition in heathland along an experimental gradient of nutrient availability. *Oikos*, 57: 310-318.

Bakker, J.P. 1989. *Nature management by grazing and cutting*. Academic Press Publishers, Dordrecht, The Netherlands.

Bakker, J.P., Podschlod, P., Strykstra, R.J., Bekker, R.M., Thompson, K. 1996. Seed banks and seed dispersal: important topics in restoration ecology. *Acta Botanica Neerlandica*, 45: 461-490.

Bekker, R., Van den Berg, L., Strykstra, R., Verhagen, R. 2005. Heidevegetaties, zo gezaaid?: Het opbrengen van maaisel als versnelde natuurontwikkelingmaatregel geëvalueerd (themanummer). *De Levende Natuur*, 106: 214-218.

Bekker, R.M., Schaminée, J.H.J., Bakker, J.P., Thompson, K. 1998. Seed bank characteristics of Dutch plant communities. *Acta Botanica Neerlandica*, 47: 15-26.

Berendse, F. 1990. Organic matter accumulation and nitrogen mineralization during secondary succession in heathland ecosystems. *Journal of Ecology*, 78: 413-427.

Bobbink, R. 1991. Effects of nutrient enrichment in Dutch chalk grassland. *Journal of Applied Ecology*, 28: 28-41.

Bobbink, R., Ashmore, M., Braun, S., Flückiger, W., Van den Wyngaert, I.J.J. 2003. Empirical nitrogen critical loads for natural and semi-natural ecosystems: 2002 update. In: Achermann, B., Bobbink, R. (Eds.), *Empirical critical loads for nitrogen - Proceedings.*

SAEFL, Berne, Switzerland, pp. 43-171.
Bobbink, R., Bik, L., Willems, J.H. 1988. Effects of nitrogen fertilization on vegetation structure and dominance of *Brachypodium pinnatum* (L.) Beauv. in chalk grassland. *Acta Botanica Neerlandica*, 37: 231-242.
Bobbink, R., Heil, G.W. 1993. Atmospheric deposition of sulphur and nitrogen in heathland ecosystems. In: R. Aerts, G.W. Heil (Eds.), *Heathlands: Patterns and Processes in a changing environment. Geobotany 20*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 25-50.
Bobbink, R., Hornung, M., Roelofs, J.G.M. 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, 86: 717-738.
Boorman, L.A., Fuller, R.M. 1982. Effects of added nutrients on dune swards grazed by rabbits. *Journal of Ecology*, 70 (1): 345-355.
Boxman, A.W., Krabbendam, H., Bellemakers, M.J.S., Roelofs, J.G.M. 1991. Effects of ammonium and aluminium on the development and nutrition of *Pinus nigra* in hydroculture. *Environmental Pollution*, 73: 119-136.
Britton, A.J., Marrs, R.H., Carey, P.D., Pakeman, R.J. 2000. Comparison of techniques to increase *Calluna vulgaris* cover on heathland invaded by grasses in Breckland, South East England. *Biological Conservation*, 95: 227-232.
Carroll, J.A. Caporn, S.J.M., Johnson, D., Morecroft, M.D., Lee, J.A. 2003. The interactions between plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition. *Environmental Pollution*, 121: 363-376.
De Graaf M.C.C., Bobbink R., Verbeek P.J.M., Roelofs, J.G.M. 1997. Aluminium toxicity and tolerance in three heathland species. *Water, Air and Soil Pollution*, 98: 229-241.
De Graaf M.C.C., Bobbink, R., Roelofs, J.G.M., Verbeek, P.J.M. 1998a. Differential effects of ammonium and nitrate on 3 heathland species. *Plant Ecology*, 135: 185-196.
De Graaf, M.C.C., Verbeek, P.J.M., Bobbink, R., Roelofs, J.G.M. 1998b. Restoration of species-rich dry heaths: the importance of appropriate soil conditions. *Acta Botanica Neerlandica*, 47: 89-113.
De Graaf M.C.C. 2000. *Exploring the Calcicole - Calcifuge gradient in heathlands*. PhD Thesis, University of Nijmegen, Nijmegen, The Netherlands.
De Graaf, M.C.C., Verbeek, P.J.M., Cals, M.J.R., Roelofs, J.G.M. 1994. *Effectgerichte Maatregelen tegen Verzuring en Eutrofiëring van Matig Mineraalrijke Heide en Schraallanden*. University of Nijmegen, Nijmegen, The Netherlands.
Dorland, E., Bobbink, R., Messelink, J.H., Verhoeven, J.T.A. 2003. Soil ammonium accumulation after sod cutting hampers the restoration of degraded wet heathlands. *Journal of Applied Ecology*, 40: 804-814.
Dorland, E., Kerkhof, A.C., Rulli, J.M., Bobbink, R. 2004. Mesocosm seepage experiment to restore the buffering capacity of acidified wet heath soils. *Ecological Engineering*, 23(3): 221-229.
Dorland, E., van den Berg, L.J.L., Brouwer, E., Bobbink, R. 2005. Catchment liming to restore degraded, acidified heathlands and moorland pools. *Restoration Ecology*, 13 (2): 302-311.
Ehrlen, J., Van Groenendael, J. 2001. Storage and the delayed costs of reproduction in the understorey perennial Lathyrus vernus. *Journal of Ecology*, 89 (2): 237-246.
Falkengren-Grerup, U. 1995. Interspecies differences in the preference of ammonium and nitrate in vascular plants. *Oecologia*, 102: 305-311.
Falkengren-Grerup, U., Lakkenborg-Kristensen, H. 1994. Importance of ammonium and nitrate to the performance of herb-layer species from deciduous forests in southern Sweden. *Environmental and Experimental Botany*, 34: 31-38.

Falkengren-Grerup, U., Diekmann, M. 2003. Use of a gradient of N-deposition to calculate effect-related soil and vegetation measures in deciduous forests. *Forest Ecology and Management*, 180 (1-3): 113-124.

Gigon, A., Rorison, I.H. 1972. The response of some ecologically distinct plant species to nitrate- and to ammonium-nitrogen. *Journal of Ecology*, 60: 93-102.

Gimmingham, C.H. 1972. *Ecology of Heathlands*. Chapman and Hall, London, United Kingdom.

Gerendás, J., Ratcliffe, R.G., Sattelmacher, B. 1990. ^{31}P nuclear magnetic resonance evidence for differences in intracellular pH in the roots of maize seedlings grown with nitrate or ammonium. *Journal of Plant Physiology*, 137: 125-128.

Güsewell, S. 2004. N:P ratios in terrestrial plants: variation and functional significance. Tansley Review. *New Phytologist*, 164: 243-266.

Heil, G.W., Diemont, W.H. 1983. Raised nutrient levels change heathland into grassland. *Vegetatio*, 53: 113-120.

Jansen, A.J.M., Roelofs, J.G.M. 1996. Restoration of Cirsio-Molinietum wet meadows by sod cutting. *Ecological Engineering*, 7: 279-298.

Jones, M.L.M. Hayes, F., Brittain, S.A. Haria, S., Williams, P.D., Ashenden, T.W., Norris, D.A. Reynolds, B. 2003. Field survey linking soil, vegetation and groundwater parameters in UK sand dunes with atmospheric nitrogen deposition. In: Achermann, B., Bobbink, R. (Eds.) *Empirical critical loads for nitrogen - Proceedings*. SAEFL, pp. 291-293.

Jones, M.L.M., Reynolds, B., Stevens, P.A., F., Norris, Emmet, B.A. 2002. *Changing nutrient budget of sand dunes: consequences for the nature conservation interest and dune management. A review.* Centre for Ecology and Hydrology. Bangor, LL57 2UP, United Kingdom.

Kinraide, T.B. 1997. Reconsidering the rhizotoxicity of hydroxyl, sulphate and fluoride complexes of aluminium. *Journal of Experimental Botany*, 48: 1115-1124.

Kirkby, EA. 1968. Influence of ammonium and nitrate nutrition on the cation-anion balance and nitrogen and carbohydrate metabolism of white mustard plants grown in diluted solutions. *Soil Science*, 105: 133-141.

Kooijman, A.M., Besse, M. 2002. The higher availability of N and P in lime-poor than in lime-rich coastal dunes in the Netherlands. *Journal of Ecology*, 90: 394-403.

Kreutzer, K. 1995. Effects of forest liming on soil processes. *Plant and Soil*, 169:447-470.

Koerselman W., Meuleman A.F.M. 1996. The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology*, 33: 1441-1450.

Lee, J.A., Caporn, S.J.M. 1998. Ecological effects of atmospheric reactive nitrogen deposition on semi-natural terrestrial ecosystems. *New Phytologist*, 139: 127-134.

Lucassen, E.C.H.E.T., Bobbink, R., Smolders, A.J.P., Van der Ven, P.J.M., Lamers, L.P.M., Roelofs, J.G.M. 2003. Interactive effects of low pH and high ammonium levels responsible for the decline of *Cirsium dissectum* (L.) Hill. *Plant Ecology*, 165: 45-52.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants* (2nd edition). Academic Press, London, United Kingdom.

Mehrer, I., Mohr, H. 1989. Ammonium toxicity: description of the syndrome in *Sinapis alba* and the search for its causation. *Physiologia Plantarum*, 77: 545-554.

Morecroft, M.D., Sellers, E.K., Lee, J.A. 1994. An experimental investigation into the effects of atmospheric nitrogen deposition on two semi-natural grasslands. *Journal of Ecology*, 82: 475-483.

Paulissen, M.P.C.P., van der Ven, P.J.M., Dees, A.J., Bobbink, R. 2004. Differential effects of nitrate and ammonium on three fen bryophyte species in relation to pollutant nitrogen input. *New Phytologist*, 164(3): 451-458.

Power, S.A., Ashmore, M.R., Cousins, D.A., Sheppard, L.J. 1998. Effects of nitrogen addition on the stress sensitivity of Calluna vulgaris. *New Phytologist*, 138: 663-673.

Roelofs, J.G.M. 1986. The effect of airborne sulphur and nitrogen deposition on aquatic and terrestrial heathland vegetation. *Experientia*, 42: 372-377.

Roelofs, J.G.M., Bobbink, R., Brouwer, E., De Graaf, M.C.C. 1996. Restoration ecology of aquatic and terrestrial vegetation on non-calcareous sandy soils in the Netherlands. *Acta Botanica Neerlandica*, 45: 517-541.

Scheffer, F., Schachtschabel, P. 1979. *Lehrbuch der Bodenkunde*. Ferdinand Enke Verlag, Stuttgart, Germany.

Smart, S.M., Ashmore, M.R., Hornung, M., Scott, W.A., Fowler, D.A., Dragosits, U., Howard, D.C., Sutton, M.A., Famulari, D. 2004. Detecting the signal of atmospheric N deposition in recent national-scale vegetation change across Britain. *Water, Air and Soil Pollution: Focus*, 4: 296-278.

Snow, C.S.R., Marrs, R.H. 1997. Restoration of Calluna heathland on a Bracken Pteridium - infested site in North West England. *Biological Conservation*, 81: 35-42.

Süß, K., Storm, C., Zehm, A., Schwabe, A. 2004. Succession in inland sand ecosystems: which factors determine the occurrence of the tall grass species *Calamagrostis epigejos* (L.) Roth and *Stipa capillata* L.? *Plant Biology*, 6(4): 465-476.

Troelstra, S.R., Wagenaar, R., De Boer, W. 1990. Nitrification in Dutch heathland soils. 1. General soil characteristics and nitrification in undisturbed soil cores. *Plant and Soil*, 127: 179-192.

Tomassen, H.B.M., Smolders, A.J.P., Lamers, L.P.M., Roelofs, J.G.M. 2003. Stimulated growth of *Betula pubescens* and *Molinia caerulea* on ombrotrophic bogs: role of high levels of atmospheric nitrogen deposition. *Journal of Ecology*, 91(3): 357-370.

Van Breemen, N., Burrough, P.A., Velthorst, E.J., van Dobben, H.F, de Wit, T., Ridder, T.B., Reijnders, H.F.R. 1982. Soil acidification from atmospheric ammonium sulphate in forest canopy throughfall. *Nature*, 299: 548-550

Van Beusichem, M.L., Kirkby, E.A., Baas, R. 1988. Influence of nitrate and ammonium nutrition on the uptake, assimilation and distribution of nutrients in *Ricinus communis*. *Plant Physiology*, 86: 914-921.

Van Dijk, H.F.G., Roelofs, J.G.M. 1988. Effects of excessive ammonium deposition on the nutritional status and condition of pine needles. *Physiology of Plants*, 73: 494-501.

Vergeer, P., Rengelink, R., Copal, A., Ouborg, N.J. 2003. The interacting effects of genetic variation, habitat quality and population size on performance of *Succisa pratensis*. *Journal of Ecology*, 91: 18-26.

Vergeer, P., Sonderen, E., Ouborg, N.J. Introduction strategies put to the test: local adaptation versus heterosis. *Conservation Biology* (in press).

Willis, A.J. 1963. Braunton Burrows: the effects on the vegetation of the addition of mineral nutrients to the dune soils. *Journal of Ecology*, 51: 353-374.

Webb, NR. 1998. The traditional management of European heathlands. *Journal of Applied Ecology*, 35: 987-990.


pH 5
NH4 1000µM

Samenvatting

NEDERLANDSE SAMENVATTING

Dit proefschrift is getiteld 'Species-rich heathlands degraded by atmospheric N deposition, perspectives for restoration', vrij vertaald: De achteruitgang van soortenrijke heiden als gevolg van atmosferische stikstof depositie, perspectieven voor herstel. Soortenrijke graslanden vertonen veel overeenkomsten met heischrale graslanden (veel overeenkomende soorten en beide hebben voedselarme bodems) en zijn derhalve ook onderzocht in dit proefschrift. Zoals in de titel beschreven hebben beide systemen te maken met een sterke achteruitgang. Deze achteruitgang is zichtbaar in zowel het totale oppervlakte, als wel in het aantal planten en diersoorten dat er leeft. Internationaal gezien zijn het unieke ecosystemen en er rust ons een taak om de resterende gebieden te beschermen en waar mogelijk de schade te herstellen. Het doel van dit onderzoek was dan ook om mogelijke knelpunten en oorzaken die het beheer en herstel van de heiden en heischrale graslanden in de weg staan op te sporen.
In dit proefschrift richt ik me op de interacties tussen de biogeochemie en de ontwikkeling van de vegetatie van droge soortenrijke graslanden en heiden. Dit houdt in dat ik gekeken heb naar de chemische eigenschappen van de bodem, die als gevolg van biologische processen veranderen (vandaar de naam biogeochemie) en de invloed die deze eigenschappen hebben op de planten die in deze bodem groeien. Specifiek heb ik gekeken naar de effecten van atmosferische stikstof depositie op de bodemchemie, de planten en de mogelijkheden voor herstel en beheer. Omdat al eerder is aangetoond dat ammonium, een belangrijke component uit de atmosferische stikstofdepositie, een sterk negatief effect kan hebben op vele plantensoorten heb ik mij geconcentreerd op de effecten van een hoge ammonium depositie op de vegetatie van droge soortenrijke graslanden en heiden.
Dit onderzoek maakt deel uit van het door de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) gesubsidieerde onderzoeksprogramma 'Stimuleringsprogramma Biodiversiteit'. Hierbinnen is ondermeer onderzoek verricht naar de knelpunten in het herstel van natte heiden en heischrale graslanden door Edu Dorland (Universiteit van Utrecht) en naar de verspreiding van zaden en de invloed van begrazing in het heidelandschap door Maarten Mouissie (Universiteit van Groningen).

OPKOMST EN ACHTERUITGANG

De meeste Nederlandse heide en heischrale graslanden zijn halfnatuurlijk. Ze zijn vaak eeuwen geleden ontstaan door traditionele landbouwpraktijken. Hierbij werden grote oppervlakten land kaalgekapt en geschikt gemaakt voor begrazing door vee. Het vee (voornamelijk schapen) liet men overdag op deze 'woeste gronden' (de heide) grazen en 's nachts werden de schapen in stallen gedreven (de zogenaamde potstallen). De mest die het vee 's nachts op stal deponeerde werd vermengd met plagsel, dat jaarlijks werd gewonnen uit de heide. De vermengde mest met het heideplagsel werd gebruikt voor de bemesting van de akkers. Door begrazing en het regelmatig plaggen bleven deze heiden en heischrale graslanden voedselarm. Eeuwenlang zorgde dit systeem voor het instant houden van enorme

oppervlakten heide met een vegetatie die was aangepast aan enige begrazing en voedselarme omstandigheden. Struikheide in de droge delen en Gewone dopheide in de natte delen domineerden de vegetatie. Voor één hectare vruchtbare akker was ongeveer tien hectare heide nodig om de aanvoer van voldoende schapenmest en heideplaggen te garanderen. Het is dan ook niet gek dat met de intrede van de kunstmest eind 19e eeuw het potstalsysteem al snel niet meer werd toegepast. Vanaf omstreeks 1900 werd de heide ontgonnen. Ontginning maar ook drainage zijn belangrijke oorzaken voor de achteruitgang van de heide en heischrale graslanden.
Een andere belangrijke oorzaak voor het verdwijnen van deze systemen is de hoge zwavel- en stikstof atmosferische depositie. De atmosferische depositie van zwavel en stikstof in Nederland is sterk toegenomen sinds de 2e helft van de twintigste eeuw, met een piek in de jaren '80. In deze periode zijn veel karakteristieke plantensoorten van de heide en het heischrale milieu (bijvoorbeeld Valkruid en Rozenkransje) achteruitgegaan of geheel verdwenen ten koste van snel groeiende soorten (voornamelijk grassen zoals Bochtige smele en Pijpestrootje). Zowel de verzuring (voornamelijk door zwaveldepositie) als vermesting (voornamelijk door stikstof depositie) hebben gezorgd voor een ernstige vergrassing en een drastische afname en soms zelfs voor het volledig verdwijnen van veel heiden en heischrale graslanden.

VERMESTING (EUTROFIËRING)

Een voorzichtige schatting geeft aan dat in 1833 er in Nederland ongeveer 600.000 hectare heide was, in 1988 was dat nog maar 42.000 hectare. In Nederland komen de restanten heide en heischrale graslanden voor op de zandgronden, op de Waddeneilanden en langs de kust in de duingebieden. Veruit de meeste van onze heidevelden en heischrale graslanden zijn zoals hierboven beschreven halfnatuurlijk, slechts de heiden uit het duinlandschap hebben een natuurlijke oorsprong. Tegenwoordig zijn de meeste heidevelden opgenomen in natuurgebieden en reservaten. Dit beschermt ze tegen ontginningen en vaak ook tegen overdadige wateronttrekking. De huidige neerslag van zwavel componenten is grotendeels teruggedrongen door gerichte maatregelen. De neerslag van ammoniak uit de landbouw is dankzij maatregelen aan het dalen (bijvoorbeeld door het verplicht injecteren van mest). De Nederlandse heiden en soortenrijke (heischrale) graslanden kampen echter nog steeds met de 'erfenis' van de jarenlange hoge stikstof belasting uit de atmosfeer. De eens zo voedselarme systemen zijn nu voedselrijk en hebben door de jarenlange hoge stikstofbelasting vaak een grote hoeveelheid stikstof opgeslagen in de bodem.
De toename van stikstof in de bodem als gevolg van de atmosferische depositie van stikstof heeft een verschuiving in de soortensamenstelling van veel soortenrijke graslanden, heischrale milieus en heiden tot gevolg. In het voedselarme milieu komen veel plantensoorten voor welke zijn aangepast aan voedselarme omstandigheden. Bij toenemende voedselbeschikbaarheid kunnen snel groeiende soorten (meestal grassen) van deze verhoogde beschikbaarheid profiteren en hierdoor de trager groeiende soorten van de voedselarme systemen overwoekeren. Dit werd onder andere ook aangetoond in een experiment waarin verschillende

bakken met duingrasland vegetatie werden beregend met verschillende hoeveelheden stikstof (**hoofdstuk 2**). Uit dit onderzoek blijkt duidelijk dat naarmate de stikstofgift hoger was, de planten ook meer stikstof opnemen en dat bij een gift van meer dan 20 kg stikstof per hectare per jaar, doelsoorten als Geel walstro en Driedistel verdwijnen ten koste van het gras Duinriet. Dergelijke resultaten zijn ook in andere graslanden en heiden waargenomen. Het is gebleken dat het verschil in competitiekracht tussen de snelgroeiende en de langzaam groeiende soorten een van de verklaringen is voor de achteruitgang van deze ecosystemen onder een hoge stikstof depositie.

VERZURING

Een andere factor die een grote invloed heeft op de soortenrijkdom van de (heischrale) graslanden en heiden, is de zuurgraad (pH) van de bodem en het vermogen om zuur te bufferen (het zogenaamde bufferend vermogen). In de bodem bevindt zich een voorraad basen welke gebonden is aan organische stof en kleimineralen. Door de aanvoer van verzurende stoffen worden deze basen, zoals calcium en magnesium, geleidelijk vervangen (gebufferd) door zuur en stikstof (voornamelijk ammonium). Zolang het zuur gebufferd wordt heeft het weinig gevolgen voor de zuurgraag van de bodem en zorgt het alleen maar voor een langzame uitputting van de basen. Dit proces wordt verlaging van de basenverzadiging genoemd. Bij een lage basenverzadiging, dus als er nog maar weinig basen beschikbaar zijn om het zuur te bufferen, kan zuur niet meer worden vervangen door basen, waardoor verzuring van de bodem optreedt. Tevens kunnen door de verlaagde basenverzadiging en de daarop volgende verzuring, giftige metalen zoals aluminium in oplossing gaan. Heide en heischrale milieus hebben veelal een lage basenverzadiging wat betekent dat de bodem slechts een beperkt bufferend vermogen heeft. Een kleine toename in de hoeveelheid zuur kan hierdoor al direct een daling van de zuurgraad van de bodem, en een toename van giftige metalen zoals aluminium tot gevolg hebben.

STIKSTOF

De totale stikstofdepositie bestaat uit een gereduceerde vorm en een geoxideerde vorm. De gereduceerde vorm in gasvorm staat bekend als ammoniak en in opgeloste vorm als ammonium. De depositie van ammoniak is nauw gecorreleerd met de landbouw. In gebieden met veel agrarische activiteiten is de depositie van gereduceerd stikstof zeer hoog. Atmosferische depositie van de geoxideerde vorm is voornamelijk gecorreleerd met verkeer en industrie. Bij de neerslag van ammonium kunnen biogeochemische processen zorgen voor een ernstige verzuring van de bodem. Ammonium kan namelijk in de bodem door micro-organismen zowel worden omgezet in nitraat, een proces dat nitrificatie heet (**hoofdstuk 5**), als worden opgenomen door planten. Bij beide processen komt zuur vrij. De atmosferische stikstofdepositie resulteert dus, naast vermesting, ook in een verzuring van de bodem. De omzetting van gereduceerd stikstof (ammonium) naar geoxideerd stikstof (nitraat) verloopt pas optimaal onder gebufferde en aërobe omstandigheden. Deze

omzetting wordt geremd wanneer de zuurgraad daalt tot onder de 4,5 (door verzuring). In verzuurde situaties zal de voorraad nitraat door opname en (andere) omzettingen langzaam uitgeput raken en niet meer worden aangevuld. De voortgaande depositie van ammonium resulteert hierbij in een stijging van de ammoniumconcentraties en dus ook in een stijging van de ammonium/nitraat verhouding in de bodem.

Uit de resultaten van **hoofdstuk 3** is gebleken dat de combinatie van hoge ammoniumconcentraties en een lage zuurgraad zeer nadelige gevolgen kan hebben voor de overleving van doelsoorten uit het heischrale milieu. Planten als Rozenkransje en Blauwe knoop prefereren een licht gebufferde bodem met een zuurgraad hoger dan 5,0. In deze bodems is nitraat een veel voorkomende stikstofvorm. In zuurdere bodems (zuurgraad 4,0-5,0), waar soorten als Struikheide en Bochtige smele nog goed groeien is stikstof voornamelijk aanwezig als ammonium. Een experiment op hydrocultuur heeft aangetoond dat de soorten van het licht gebufferde milieu zeer gevoelig zijn voor toenemende ammoniumconcentraties (**hoofdstuk 3**). De biomassa van deze planten nam dramatisch af en de sterfte nam toe bij hogere ammoniumconcentraties. Deze negatieve effecten van ammonium werden versterkt wanneer de zuurgraad verlaagd werd. Soorten van de zuurdere milieus bleken niet of nauwelijks gevoelig te zijn voor hoge ammoniumconcentraties in combinatie met een lage zuurgraad. In verzuurde heiden en heischrale graslanden is het duidelijk dat hoge ammoniumconcentraties funest kunnen zijn voor de meest bedreigde soorten. De combinatie van hoge atmosferische depositie van gereduceerd stikstof (ammonium en ammoniak) en verzuring van de bodem blijkt meer negatieve gevolgen te hebben dan de atmosferische depositie van gereduceerd stikstof of verzuring alleen.

Omdat de depositie van gereduceerd stikstof (ammoniak en ammonium) voornamelijk samenhangt met de landbouw en de depositie van geoxideerd stikstof (o.a. nitraat) met industrie en verkeer is het haast wel vanzelfsprekend dat de verhoudingen in stikstofdepositie tussen gereduceerd en geoxideerd stikstof regionaal en internationaal sterk verschillen. In de meeste delen van Nederland komt de stikstofdepositie voornamelijk neer in de gereduceerde (ammoniak en ammonium) vorm. In sommige streken in Nederland en ook in andere landen komt stikstof voornamelijk in de geoxideerde vorm neer. Om te onderzoeken wat het effect is van geoxideerd of gereduceerd stikstof op een soortenrijke heide vegetatie, werden bakken met daarin een soortenrijke heidevegetatie drie jaar lang beregend met verschillende verhoudingen van gereduceerd (ammonium) en geoxideerd (nitraat) stikstof (**hoofdstuk 4**). Daarnaast werd de helft van deze bakken eenmalig bekalkt om het effect van de zuurgraad en basenverzadiging van de bodem te onderzoeken in relatie tot deze ammonium/nitraatverhoudingen. Het bleek dat hoge verhoudingen ammonium/nitraat (dus veel ammonium en weinig nitraat) gepaard ging met een sterke afname in het soortenaantal en dat in het bijzonder de bedreigde, zuurgevoelige soorten uit deze milieus verdwenen. De groei en overleving van de soorten Rozenkransje, Valkruid en Klokjesgentiaan nam aanzienlijk af bij hoge ammonium/nitraat verhoudingen onder zure, niet gebufferde omstandigheden. In de bakken waar kalk aan werd toegevoegd werd een verhoogde overleving van deze

soorten gevonden. Soorten van de zuurdere milieus bleken niet gevoelig voor hoge ammonium/nitraat verhoudingen. In de bakken die beregend werden met lage ammoniumconcentraties en hoge nitraatconcentraties bleken zowel de zuurtolerante als de zuurintolerante soorten te overleven. Dit toont aan dat voornamelijk stikstof in de ammoniumvorm een negatief effect heeft op het voorkomen van bedreigde soorten in de Nederlandse heiden. Temeer werd aangetoond dat deze negatieve gevolgen van ammonium beter zichtbaar waren in het zure milieu en dat de planten minder last van ammonium hadden in het licht gebufferde milieu.

MAATREGELEN TEGEN VERZURING EN VERMESTING

In Nederland zijn sinds de jaren 80 van vorige eeuw een groot aantal herstelmaatregelen gestart om de negatieve effecten van verzuring en vermesting tegen te gaan. In heiden en het heischrale graslanden is plaggen een veel toegepaste maatregel tegen de vergrassing. Hierbij wordt de vegetatie samen met de bovenste (voedselrijke) toplaag van de bodem weggenomen waardoor een voedselarme kale bodem overblijft. De heide vegetatie kan zich vanuit de zaadbank weer opnieuw vestigen zoals te zien is in **figuur 1**.

Figuur 1: Droge heide in de Schaopedobbe, Friesland, gedomineerd door Struikheide drie jaar na plaggen.

De successen van plaggen zijn wisselend en lang niet altijd keren alle soorten van de heiden en heischrale graslanden terug. Een van de oorzaken hiervoor is een enorme ammoniumophoping in de eerste jaren na plaggen (**hoofdstuk 5**). Zoals gebleken is kan ammonium in hoge concentraties toxisch zijn voor veel bedreigde plantensoorten. Veel van deze soorten zullen, als ze aanwezig zijn in de zaadbank,

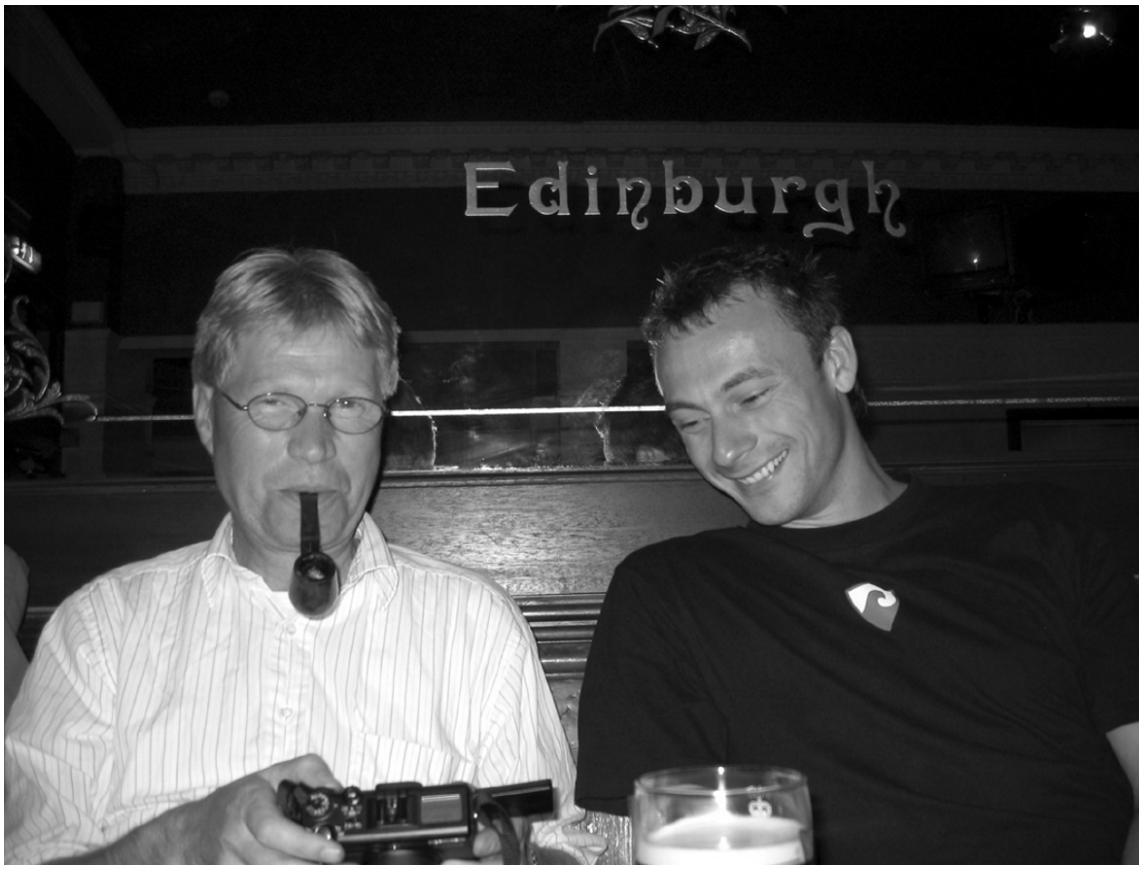
direct na kieming sterven als gevolg van deze hoge concentraties. Daar de bedreigde soorten vaak kruiden zijn met een kort levende zaadbank heeft de sterfte van deze soorten vaak het verdwijnen van de soorten uit de zaadbank en dus uit het gebied tot gevolg.
De ammoniumophoping werd in zowel droge als natte heiden waargenomen na plaggen. Één van de belangrijke oorzaken van deze ophoping van ammonium is een sterk verminderde omzetting van ammonium naar nitraat door bacteriën (**hoofdstuk 5**). Omdat we met plaggen ook een deel van de verantwoordelijke bodembacteriën weghalen én door ongunstige bodemcondities zoals een te lage zuurgraad en een te laag vochtgehalte na plaggen (**hoofdstuk 6**), werd er na plaggen nauwelijks nog nitrificatie gemeten. Het toevoegen van kalk zorgde voor een stijging van zuurgraad in de bodem en daarmee werd de nitrificatie gestimuleerd. In bodems waaraan kalk werd toegevoegd werd dan ook veel minder ammonium gemeten als gevolg van een hogere nitrificatie, waardoor ammonium beneden de toxische concentratieniveaus bleef.
Met het afvoeren van de bovenste, organische (voedselrijke) laag van de bodem worden alle organische delen weggenomen en blijft een voedselarme, minerale bodem achter. Zoals hierboven beschreven werd daarmee een groot deel van de micro-organismen verwijderd die zorg dragen voor belangrijke bacteriële omzettingen in de bodem. Echter, gebleken is dat met het verwijderen van het organisch materiaal ook een groot deel van het bufferend vermogen van de bodem wordt verwijderd (**hoofdstuk 6**). De organische toplaag van de bodem bevat humuszuren, welke complexen kunnen vormen met vrije ionen waardoor deze 'geneutraliseerd' worden. Dit is vooral belangrijk in verzuurde situaties omdat in deze situaties aluminium in oplossing kan raken. Van aluminium is gebleken dat het zeer giftig is voor veel soorten. Onderzoek heeft aangetoond dat het voorkomen van bedreigde plantensoorten uit de soortenrijke graslanden en de heischrale milieus zeer nauw gecorreleerd is met de concentraties aluminium en calcium. Hoge aluminiumconcentraties zijn zeer toxisch voor bedreigde plantensoorten. Echter, als de calciumconcentraties ook hoog zijn dan worden veel van deze negatieve effecten teniet gedaan. In **hoofdstuk 6** is aangetoond dat met het verwijderen van de bovenste toplaag (en dus de humuszuren) de kieming en overleving van de bedreigde doelsoort Valkruid dramatisch afneemt. Tevens is aangetoond dat de plagdiepte een rol kan spelen; hoe dieper men plagt hoe meer humuszuren men weghaalt en hoe lager de kieming en overleving van Valkruid. De verlaging van de overleving als gevolg van het plaggen en daarmee het verwijderen van humuszuren blijkt gerelateerd te zijn aan aluminiumtoxiciteit. Bekalken van de geplagde delen heeft ook hier weer een positief effect in die zin dat de concentraties calcium toenemen en daarmee de aluminium/calcium ratio in de bodem gunstiger wordt voor kieming en vestiging van doelsoorten.

TOEKOMST VAN DE HEIDE EN HEISCHRALE GRASLANDEN

De ammoniumdepositie in Nederland is langzaam aan het dalen. Dit houdt in dat de verhouding van gereduceerd versus geoxideerd stikstof dat neerslaat in ons land aan het veranderen is; was het in de jaren tachtig nog 80 tot 90 procent gereduceerd

stikstof, is het nu 50 tot 60 procent gereduceerd stikstof. Dit is een belangrijk gegeven met het oog op de herstelmaatregelen voor de (Nederlandse) heide. Zoals boven beschreven zijn voornamelijk ammoniak en ammonium verantwoordelijk voor de afname van soorten en een toename van de vergrassing. Een zeer sterke verzuring door atmosferische depositie valt dan ook nu niet meer te verwachten daar de zwaveldepositie sterk is terug gedrongen door zeer effectieve maatregelen en de atmosferische depositie van gereduceerd stikstof nu aan het dalen is. Uit onderzoek is gebleken dat de pH en basenverzadiging van verzuurde heiden en vennen zeer goed te herstellen zijn door eenmalig bekalken. De effectiviteit van deze maatregel bleek zeer hoog en ten minste voor 15 jaar zichtbaar. Met de huidige atmosferische depositie verschuivingen zal de duurzaamheid van een dergelijke maatregel alleen maar langer worden. Het is zeer waarschijnlijk dat eenmalig bekalken voldoende is om de zuurlast uit het verleden te neutraliseren.
Plaggen is een succesvolle maatregel gebleken als het gaat om het terugdringen van de vergrassing. Veelal wordt er geplagd in verzuurde, sterk vergraste heiden en heischrale milieus. Het risico op een ammoniumophoping en hoge aluminium concentraties als gevolg van het plaggen in deze milieus is hoog. Een combinatie van plaggen en eenmalig bekalken lijkt een goede en duurzame maatregel die het herstel en behoud van bedreigde plantensoorten stimuleert.

Voor een leuke en recente beschrijving van de stand van zaken over het herstel van de Nederlandse heide verwijs ik u graag naar het themanummer 'Herstel van de Heide' van *De Levende Natuur*, nummer 106 (5), september 2005. Verkrijgbaar via **http://www.delevendenatuur.nl/**


Edinburgh

Dankwoord

DANKWOORD

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Leon

Curriculum vitae

CURRICULUM VITAE

Ik ben geboren op 8 november 1974 te Helmond. Na het VWO begon ik in 1993 aan de studie biologie aan de (toen nog) Katholieke Universiteit Nijmegen. Ik specialiseerde me in de Algemene Ecologie en liep twee hoofdvak-stages. Eén bij de afdeling Aquatische Ecologie en Milieubiologie bij Dr. Joop Ouborg waarin ik onderzoek deed naar de klonale voortplanting van de Slijkzegge (*Carex limosa*) in drijftillen in Connemara te Ierland en één bij Dr. James Bullock op het Institute for Terrestrial Ecology in Furzebrook te Engeland. Deze laatste stage, waarbij ik onderzoek deed naar de habitatkeuze van de Provençaalse grasmus (*Sylvia undata*) op de Engelse Heide in Dorset, werd begeleid door Prof. dr. Kees Blom van de afdeling Experimentele Plantenecologie. Tijdens mijn studie heb ik diverse cursussen geassisteerd. In 1999 rondde ik mijn studie biologie *cum laude* af en in datzelfde jaar werd ik aangenomen bij de afdeling Aquatische Ecologie en Milieubiologie om in opdracht van het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) gegevens van oppervlaktewateren te verzamelen. In 2000 werd ik aangesteld als Assistent In Opleiding bij dezelfde afdeling waarvan de resultaten van het onderzoek in dit proefschrift zijn gebundeld. Tussen 2000 en 2005 gaf ik begeleiding aan verschillende research- en doctoraalstudenten. In december 2004 heb ik samen met Philippine Vergeer een symposium over het herstel van de Nederlandse heide georganiseerd. Dit heeft mede geleid tot het uitkomen van een themanummer van *De Levende Natuur*, waarvan ik ook gast-redactielid was. Sinds 2005 ben ik als research fellow werkzaam aan de Environment Department van de Universiteit van York, Engeland, bij Prof. dr. Mike Ashmore en bij de afdeling Aquatische Ecologie en Milieubiologie van de Radboud Universiteit Nijmegen bij Prof. dr. Jan Roelofs.

INTERNATIONAL PUBLICATIONS

Dorland, E., Van den Berg, L.J.L., Brouwer, E., Roelofs, J.G.M., Bobbink, R. 2005 Catchment liming to restore degraded, acidified heathlands and moorland pools. *Restoration Ecology*, 13 (2): 302-311.

Van den Berg, L.J.L., Thomassen, H.B.M., Roelofs, J.G.M., Bobbink R. 2005. Effects of nitrogen enrichment on coastal dune grassland: a mesocosm study. *Environmental Pollution*, 138: 77-85.

Van den Berg, L.J.L., Dorland, E., Vergeer, P., Hart, M.A.C., Bobbink, R., Roelofs J.G.M. 2005. Decline of acid-sensitive plant species in heathland can be attributed to ammonium toxicity in combination with low pH. *New Phytologist*, 166: 551-564.

Van den Berg, L.J.L., Peters, C.P.H., Roelofs, J.G.M. Reduced versus oxidised nitrogen; implications for the development of dry heathland vegetation, based on a mesocosm study (*to be submitted*).

Vergeer, P., Van den Berg, L.J.L., Baar, J., Ouborg, N.J., Roelofs, J.G.M. 2005. The effect of turf cutting on plant and mycorrhizal spore recolonisation: implications for heathland restoration. *Biological Conservation* (*in press).*

Vergeer, P., Van den Berg, L.J.L., Ouborg, N.J., Roelofs, J.G.M. 2005. Single-source versus multi-source introductions. *Plant Biology* (*under revision*).

Dorland, E., Van den Berg, L.J.L., Van de Berg, A.J., Vermeer, M.L., Roelofs, J.G.M., Bobbink, R. 2004. The effects of sod cutting and additional liming on potential net nitrification in heathland soils. *Plant and Soil*, 265: 267-277.

Van den Berg, L.J.L., Vergeer, P., Roelofs, J.G.M. 2003. Heathland restoration in the Netherlands: effects of turf cutting depth on germination of *Arnica montana*. *Applied Vegetation Science*, 6: 116-124.

Van den Berg, L.J.L., Bullock, J.M., Clarke, R.T., Langston, R.H.W., Rose, R.J. 2001. Territory selection by the Dartford warbler (*Sylvia undata*) in Dorset, England: the role of vegetation type, habitat fragmentation and population size. *Biological Conservation*, 101: 217-228.

NATIONAL PUBLICATIONS

Bekker, R., Van den Berg, L., Strykstra, R., Verhagen, R. 2005. Heidevegetaties, zo gezaaid?: Het opbrengen van maaisel als versnelde natuurontwikkelingmaatregel geëvalueerd (themanummer). *De Levende Natuur*, 106: 214-218.

Van den Berg, L., Roelofs, J. 2005. Veranderingen in samenstelling van atmosferische stikstofdepositie; effecten op de Nederlandse heide (themanummer). *De Levende Natuur*, 106: 190-192.

Van Tooren, B., Bobbink, R., Bekker, R., Van den Berg, L., Knevel, I., Schimmel, I. 2005. Nog lange weg te gaan voor volledig herstel heide (themanummer). *De Levende Natuur*, 106: 238-242.

Dorland, E., Van den Berg, L., Bobbink, R., Roelofs, J. 2003. Bekalking bij het herstel van gedegenereerde heiden en heischrale graslanden. *De Levende Natuur*, 104: 144-148.